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(54) Title: VECTORS HAVING ENHANCED EXPRESSION, AND METHODS OF MAKING AND USES THEREOF

(57) Abstract

Disclosed and claimed are vectors having enhanced expression and methods for making and using them. Enhancement of expression is from substantially co-temporal expression of at least one first nucleic acid molecule and at least one second nucleic acid molecule. The second nucleic acid molecule encodes a transcription factor or a translation factor or a transcription factor and a translation factor. The contemporaneous expression can be from operably linking the first and second nucleic molecules to a single promoter, or from operably linking the first nucleic acid molecule to a first promoter and the second nucleic molecule to a second promoter wherein the first and second promoters function substantially contemporaneously. Thus, the first and second nucleic acid molecules can be at the same locus in the vector, or at different loci. The second nucleic acid molecule can encode: one transcription factor or more than one transcription factor, or one translation factor or more than one translation factor; or at least one transcription factor and at least one translation factor. The transcription factor can be from vaccinia H4L, D6, A7, G8R, A1L, A2L, H5R, or combinations thereof. The translation factor can be from a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, or combinations thereof. The vector can be a poxvirus such as an attenuated poxvirus, e.g., NYVAC, or ALVAC.

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TITLE OF THE INVENTION

VECTORS HAVING ENHANCED EXPRESSION,
AND METHODS OF MAKING AND USES THEREOF

RELATED APPLICATIONS

5 Reference is made to the concurrently filed application of Tartaglia et al., "Vectors Having Enhanced Expression, And Methods of Making and Uses Thereof", Serial No. 08/815,809, incorporated herein by reference. Reference is also made to the copending applications of
10 Paoletti et al., USSN 08/417,210, 08/303,275, 08/709,209, 08/184,009 (incorporating by reference USSN 07/805,567, from which U.S. Patent No. 5,378,457 issued) and 08/521,016 and to U.S. Patents Nos. 5,378,457, 5,225,336, 5,453,364, 5,494,807, 5,505,941, and 5,110,587, all of
15 which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to enhanced vectors, and methods for making and using them. The vectors can have enhanced transcription or translation or
20 enhanced transcription and translation and/or expression, e.g., enhanced transcription or translation or transcription and translation and/or expression from a nucleotide sequence of interest.

Several publications are referenced in this
25 application. Full citation to these publications is found where cited or at the end of the specification, immediately preceding the claims or where the publication is mentioned; and each of these publications is hereby incorporated by reference. These publications relate to the state of the art to which the invention pertains; however, there is no admission that any of these publications is indeed prior art.

BACKGROUND OF THE INVENTION

DNA such as plasmids or naked DNA, and other
35 vectors, such as viral vectors, e.g., vaccinia virus and more recently other poxviruses, have been used for the insertion and expression from foreign genes. The basic

technique of inserting foreign genes into live infectious poxvirus involves recombination between pox DNA sequences flanking a foreign genetic element in a donor plasmid and homologous sequences present donor plasmid and homologous 5 sequences present in the rescuing poxvirus (Piccini et al., 1987). Recombinant poxviruses are constructed in steps known as in or analogous to methods in U.S. Patent Nos. 4,769,330, 4,772,848, 4,603,112, 5,505,941, and 10 5,494,807, incorporated herein by reference. A desire in vector development is attenuated vectors, e.g., for enhanced safety; for instance, so that the vector may be employed in an immunological or vaccine composition.

For instance, the NYVAC vector, derived by deletion of specific virulence and host-range genes from 15 the Copenhagen strain of vaccinia (Tartaglia et al., 1992) has proven useful as a recombinant vector in eliciting a protective immune response against an expressed foreign antigen. Likewise, the ALVAC vector, a vaccine strain of canarypox virus, has also proven 20 effective as a recombinant viral vaccine vector (Perkus et al., 1995). In non-avian hosts, both these vectors do not productively replicate (with some exceptions as to NYVAC). Since all poxviruses replicate in the cytoplasm and encode most, if not all of the proteins required for 25 viral transcription (Moss 1990), appropriately engineered foreign coding sequences under the control of poxvirus promoters are transcribed and translated in the absence of productive viral replication.

It would be an improvement over the state of 30 the art to provide enhanced vectors, e.g., vectors having enhanced transcription or translation or transcription and translation and/or expression, for instance such vectors which are attenuated; especially since attenuation may raise issues of expression levels and/or 35 persistence, and it would be an advancement to address such issues.

OBJECTS AND SUMMARY OF THE INVENTION

Recent studies on vaccinia replication have revealed certain poxvirus-encoded functions which play a role in the regulation of viral transcription and 5 translation (reviewed in Moss, 1990; Moss, 1992). Some of these vaccinia encoded functions (e.g., E3L, K3L, H4L, and combinations thereof) have now surprisingly been utilized to increase the levels and persistence of gene expression (e.g., foreign gene expression) in vectors 10 (e.g., the NYVAC and ALVAC vectors); and, are exemplary of the inventive vectors and methods.

Objects of the present invention may include at least one of: providing a method for increasing transcription or translation or transcription and 15 translation and/or expression from at least one nucleotide sequence of interest by a vector, such as a coding nucleotide sequence by a vector; a vector having enhanced transcription or translation or transcription and translation; providing a method for preparing a 20 vector having enhanced transcription or translation or transcription and translation and/or expression; providing a method for enhancing transcription or translation or transcription and translation and/or expression from a vector; providing an improved vector, 25 such as poxvirus vectors, e.g., improved NYVAC, ALVAC or TROVAC vectors; and, products therefrom.

The invention thus provides a vector for enhanced expression of at least one first nucleotide sequence. The vector is modified to comprise at least 30 one second nucleotide sequence encoding a transcription factor or translation factor or a transcription factor and a translation factor. The vector also can be modified to comprise the first nucleotide sequence. There is substantially co-temporal or substantially 35 contemporaneous expression from the first and second nucleotide sequences. The expression is in a cell having a particular phenotype, and preferably the expression of

the first and second nucleotide sequences is with respect to the phenotype of the cell. Thus, expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or
5 translation or transcription and translation.

The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter, and the first and second promoters are preferably functional
10 substantially co-temporally or contemporaneously. Thus, the first and second nucleotide sequences can be at different loci within the vector. The first and second nucleotide sequences also can be at the same locus within the vector, using the first and second promoters; or, by
15 the first nucleotide sequence and the second nucleotide sequence being operably linked to a promoter.

The transcription factor can be of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from an open reading frame selected from
20 the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, and combinations thereof. The translation factor can effect inhibition of eIF-2 α phosphorylation or inhibition of PKR phosphorylation or otherwise sequester dsRNA which actually increases the concentration required to activate
25 PKR. The translation factor can be selected from the group consisting of: a K3L open reading frame, an E3L open reading frame, a viral associated RNA I (VAI), an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, and combinations thereof.

30 The first nucleotide sequence can be exogenous, e.g., encoding an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, a fusion protein or combinations thereof.

35 The vector can be a recombinant virus, such as a poxvirus; for instance, an orthopoxvirus or an avipoxvirus, e.g., a vaccinia virus, a fowlpox virus, a

canarypox virus; preferably an attenuated virus such as an attenuated poxvirus, e.g., NYVAC, ALVAC, or TROVAC.

The invention further provides a method for preparing a inventive vector comprising modifying the 5 vector to comprise the at least one second nucleotide sequence. The method can also include modifying the vector so that it comprises at the at least one first nucleotide sequence. Preferably the vector is so modified that there is substantially co-temporal or 10 contemporaneous expression of the first and second nucleotide sequences; and, more preferably, the vector is also so modified that the expression is with respect to the phenotype of the cell. The method can comprise operably linking the first nucleotide sequence 15 to a first promoter and the second nucleotide sequence to a second promoter, wherein the first and second promoters are functional substantially co-temporally or contemporaneously. The method can also comprise operably linking the first and second nucleotide sequences to a 20 promoter.

The invention further provides an immunological, vaccine or therapeutic composition comprising at least one inventive vector and a pharmaceutically acceptable carrier or diluent.

25 The invention even still further provides a method for generating an immunological or therapeutic response in a host (animal, human, vertebrate, mammal, etc.) comprising administering to the host at least one inventive composition.

30 The invention additionally provides a method for increasing expression of at least one first nucleotide sequence by a vector comprising the first nucleotide sequence. The method comprises modifying the vector to comprise at least one second nucleotide 35 sequence encoding a transcription factor or a translation factor or a transcription factor and a translation factor. There is preferably substantially co-temporal or

contemporaneous expression of the first and second nucleotide sequences. Expression can be in a cell having a particular phenotype; and it is more preferred to have expression be with respect to the phenotype of the cell.

5 Expression of the second nucleotide sequence enhances expression of the first nucleotide sequence by enhancing transcription or translation or transcription and translation. The method can additionally comprise modifying the vector to comprise the first nucleotide

10 sequence of interest.

The invention in yet another embodiment provides a method for expressing at least one gene product *in vitro* comprising infecting, or transfecting, a suitable cell with at least one inventive vector. The

15 products therefrom can be an immunogen or epitope of interest, which can be useful in formulating therapeutic, immunological or vaccine compositions; or, for generating antibodies such as monoclonal antibodies; or, in assays, kits, tests and the like, such as diagnostic

20 compositions, e.g., for detection of antibodies.

Thus, the invention can provide compositions and methods for *in vitro* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for

25 producing a gene product (which can be used as an immunogen or epitope in a therapeutic, immunological or vaccine composition, or in a diagnostic or detection kit, assay or method, e.g., to ascertain the presence or absence of antibodies, or to generate antibodies, such as

30 monoclonal antibodies, e.g., for use in a diagnostic or detection kit, assay or method), and/or for *ex vivo* transcription or translation or transcription and translation and/or expression involving at least one inventive vector, e.g., methods for producing a gene

35 product for stimulating cells for reinfusion into a host (e.g., animal, mammal, vertebrate, human).

Additionally, in a further embodiment the

invention provides a method for expressing at least one nucleotide sequence (e.g., the at least one first nucleotide sequence) *in vivo* comprising administering at least one inventive vector to a host (human, animal, 5 vertebrate, mammal, etc.). The nucleotide sequence can encode an immunogen or epitope of interest. The method can obtain antibodies. From generating antibodies one can generate monoclonal antibodies; or, antibodies are useful in assays, kits, tests or diagnostic compositions, 10 e.g., for detection of antigens.

The invention can thus provide methods and compositions for *in vivo* transcription or translation or transcription and translation and/or expression involving the inventive vectors, e.g., administering at least one 15 inventive vector or a composition comprising at least one inventive vector, for instance, therapeutic, immunological or vaccine compositions comprising at least one inventive vector and a suitable carrier or diluent (e.g., suitable for veterinary and human medicine).

20 These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by 25 way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

Fig. 1 shows the nucleotide sequence of the 30 insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter (SEQ ID NO:);

Fig. 2 shows the nucleotide sequence of the ALVAC C8 Insertion site containing the H6/H42 expression cassette (SEQ ID NO:);

35 Fig. 3 shows the nucleotide sequence of the ALVAC C6 insertion site containing the H6/K3L and E3L expression cassette (SEQ ID NO:);

Fig. 4 shows the DNA sequence of the coding region of FHV gB with modified T5NT motifs (SEQ ID NO:);

5 Fig. 5 shows the DNA sequence of the H6 promoted FHV gB donor plasmid pC3H6FHVB (SEQ ID NO:);

Figs. 6 and 7 show DNA and amino acid sequences (SEQ ID NOS:) of inserts in vCP1433 and vCP1452; and

Fig. 8 shows the DNA sequence (SEQ ID NO:) of K3L E3L in vCP1452.

10 **DETAILED DESCRIPTION**

U.S. Patent No. 5,494,807, to Paoletti et al., hereby incorporated herein by reference, relates to a modified recombinant virus having inactivated virus-encoded genetic functions so that the recombinant virus 15 has attenuated virulence and enhanced safety. The viruses disclosed in Paoletti et al. can be poxviruses, e.g., a vaccinia virus or an avipox virus, such as fowlpox virus and canarypox virus, e.g., NYVAC, ALVAC and TROVAC. ALVAC was deposited under the terms of the 20 Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, 20852, USA, ATCC accession number VR-2547. TROVAC was likewise deposited under the terms of the Budapest Treaty with the ATCC, accession number 2553. And, NYVAC (vP866), vP994, 25 vCP205, vCP1433, placZH6H4Lreverse, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers ___, ___, ___, ___, ___, and ___, respectively.

Like the Paoletti et al. issued U.S. Patent, 30 Falkner et al., WO 95/30018, published November 9, 1995, based on U.S. application 08/235,392, filed April 24, 1994 (both incorporated herein by reference), relates to poxviruses wherein loci for genetic functions associated with virulence (i.e., loci for "essential" functions) are 35 employed for insertion of exogenous DNA.

Further, recombinants can be made from early (DNA⁻) and late defective mutants (see Condit and Niles,

"Orthopoxvirus Genetics," pp 1-39, In: Poxviruses, Edited by R. W. Moyer and P. C. Turner (Springer-Verlag, 1990), and documents cited therein, hereby incorporated herein by reference)), or from MVA which is said to be abortive late. Recombinants from defective mutants, abortive late viruses, viruses having essential genetic functions deleted or interrupted, or viruses having expression without productive replication (e.g., ALVAC in mammalian systems) may be said to be attenuated.

Certain vectors, such as attenuated vectors, e.g., NYVAC and ALVAC vectors, are blocked or limited in late gene expression in mammalian cells. Thus, early promoters are routinely employed in such vectors, e.g., NYVAC- or ALVAC-based recombinants, for expression from the foreign gene products.

Vaccinia encodes an open reading frame (ORF) designated H4L which has been shown to be required for early viral transcription (Ahn and Moss 1992, Zhang et al, 1994). The H4L ORF encodes an essential protein of 94 kDa which is expressed after the start of viral DNA replication (late function). The H4L protein has been found to be tightly associated with the viral RNA polymerase complex and is believed to act in conjunction with the vaccinia early transcription factor (VETF) to initiate and transcribe early viral message (Ahn and Moss, 1992).

H4L is expressed late, but required early. This is consistent with the protein being packaged in the viral particles similar to that which is observed with VETF. This suggested that the amount of H4L present at early times post infection is low and perhaps limiting. Hence, one approach to increase foreign gene expression in an abortive, early vector-, e.g., virus-host interaction would be to increase the amount of H4L protein available during the early phase by expressing the H4L ORF using a vaccinia early/late promoter rather than the endogenous late promoter. Early expression from

H4L may not only increase the level of foreign gene transcripts, but also increase levels of other vaccinia early genes (e.g. E3L) which may also increase total protein levels.

5 There are other viral transcription factors; for instance, early and/or late viral transcription factors of poxvirus origin; e.g., from: vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, or vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X; see Kovacs
10 et al., J. Virology, October 1996, 70(10):6796-6802, and documents cited therein, incorporated herein by reference). These and other transcription factors, and nucleotide sequences therefor or for homologs thereof, e.g., from another poxvirus, are useful in the practice
15 of the invention.

 The selection of a suitable transcription factor is within the ambit of the skilled artisan from this disclosure and knowledge in the art; for instance, the skilled artisan can select a transcription factor
20 based on an abortive phenotype of the vector, e.g., MVA is said to be abortive late, and a late or early or early/late transcription factor may be employed with this vector; ALVAC is abortive early and an early or early/late transcription factor may be employed with this
25 vector; and, the vector can also be a ts (temperature sensitive) mutant (with respect to early (DNA⁻) and late defective mutants which can be also used in the practice of this invention, reference is made to Condit and Niles,*supra*). Thus, it is preferred that the transcription
30 and/or translation factor and the at least one nucleotide sequence of interest be expressed early, late (including intermediate), or early/late, relative to the phenotype of the vector.

 Another means to increase foreign gene expression involves enhancing the overall efficiency of translation, e.g., mRNA translation, such as viral mRNA translation. Two vaccinia encoded functions (E3L and

K3L) have recently been identified as playing a role in the regulation of viral translation (Beattie et al., 1995a, 1995b, 1991; Chang et al., 1992; Davies et al., 1993). Both are capable of inhibiting the action of a 5 cellular protein kinase (PKR) which, when activated by double stranded RNA (dsRNA), phosphorylates the translational initiation factor eIF-2 α , leading to an inhibition of initiation of mRNA translation (reviewed in Jacobs and Langland, 1996). Vaccinia virus, which 10 produces dsRNA during viral transcription, has thus evolved mechanisms to block the negative action of PKR on eIF-2 α and allow for efficient translation of viral mRNA. (Asymmetric transcription gives rise to dsRNA; any viral infection or plasmid derived expression gives rise to it; 15 dsRNA activates PKR; PKR becomes autophosphorylated, leading to phosphorylation of eIF-2 α .)

The vaccinia K3L ORF has been shown to have significant amino acid homology to eIF-2 α (Goebel et al., 1990; Beattie et al., 1991; U.S. Patent No. 5,378,457; 20 see also Beattie et al., 1995a, 1995b). This protein is believed to act as a pseudosubstrate for PKR and competes for the eIF-2 α binding site (Carroll et al., 1993; Davies et al., 1992). The K3L gene product can bind to 25 activated PKR and thus prevent phosphorylation of eIF-2 α with its resultant negative effect on translation initiation.

The vaccinia E3L gene codes for a protein which is capable of specifically binding to dsRNA (Watson and Jacobs, 1991; Chang et al., 1992). This would tend to 30 lower the amounts of dsRNA in the infected cell, and thus reduce the level of activated PKR. When E3L was deleted from vaccinia, the resulting virus lost this kinase inhibitory function and further allowed activation of the 2' 5' oligoadenylylate synthetase/RNase L pathway resulting 35 in increased degradation of rRNA (Beattie et al., 1995a, 1995b). Thus, E3L appears to be critical for efficient mRNA translation in vaccinia infected cells at two

levels; mRNA stability and limiting eIF-2 α phosphorylation.

The ALVAC genome has been sequenced and searched for any homology to E3L/K3L or to any known 5 dsRNA binding motif. Results have revealed no significant homology of any ALVAC ORFS to these two vaccinia ORFs, nor the presence of any dsRNA binding motifs.

Thus, an approach to improving expression 10 levels in recombinant ALVAC vectors was to express the vaccinia E3L/K3L ORFs in ALVAC under the control of early vaccinia promoters. Through inhibition of PKR in the infected cells, the levels and persistence of foreign gene expression could be enhanced.

Hence, NYVAC and ALVAC recombinants as 15 discussed herein were generated in order to enhance foreign gene expression at the transcriptional or translational or transcriptional and translational levels, as examples of the vectors and methods of the 20 present invention.

Thus, exemplified herein is NYVAC recombinants having an early expressed H4L ORF and ALVAC recombinants having expression from the vaccinia E3L/K3L genes for enhancing or increasing the levels or persistence of 25 expression of an inserted foreign gene. The up-regulation of foreign gene expression can have a profound effect on the induction of a therapeutic or immunological response in a host administered or inoculated with recombinants derived from these new vectors, thereby 30 leading to an enhanced immunological, e.g., protective, response, or an enhanced therapeutic response.

The scope of the invention, i.e., to manipulate expression from any of a transcription and/or translation factor, e.g., H4L, E3L and K3L, to thereby enhance 35 transcriptional or translational or transcriptional and translational and/or expression efficiency, can be extended to other eukaryotic vector systems (i.e. DNA,

viruses).

In fact, viruses in other families have also evolved mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation. In adenoviruses, the VAI RNA, transcribed by RNA pol III, has been well characterized and shown to bind directly to PKR, and thus, prevent its activation by dsRNA (Mathews and Shenk, 1991). Deletion of VAI from the adenovirus genome results in a mutant that replicates poorly and is deficient in levels of late gene expression (Thimmappaya et al., 1982). Similarly, Epstein-Barr virus, a herpesvirus, has an analogous RNA, called EBER, which also acts to prevent PKR activation by directly binding to the kinase (Clark et al., 1991; Sharp et al., 1993). The reovirus sigma 3 gene product has been shown to act in a similar manner as vaccinia E3L in binding dsRNA and thus preventing activation of PKR (Imani and Jacobs, 1988; see also Beattie et al. 1995a). Indeed, one study has shown that the reovirus sigma 3 gene can partially compensate a vaccinia recombinant deleted of E3L (Beattie et al., 1995a). Further, a cellular protein activated upon HIV infection (TRBP) has been shown to inhibit the activity of PKR (Park et al., 1994).

Thus, the present invention broadly relates to manipulation of expression, preferably by employing at least one transcription factor, e.g., at least one early and/or late viral transcription factor, or at least one translation factor, e.g., a nucleotide sequence encoding a product for overcoming the cellular anti-viral response of translational down-regulation through PKR activation in any eukaryotic vector system, or at least one transcription factor and at least one translation factor; for instance, to increase or enhance expression. And, the invention can pertain to any vector system, including, plasmid or naked DNA vectors, viral vectors, such as poxvirus, adenovirus, herpesvirus, baculovirus, and the like. Thus, the nucleotide sequences can be RNA

or DNA, for instance, as is suitable in view of the vector system.

Accordingly, the invention can relate to a vector modified to comprise at least one nucleotide sequence encoding at least one transcription factor, at least one translation factor, or at least one transcription factor and at least one translation factor; a method for increasing transcription and/or translation and/or expression by a vector or for preparing an inventive vector, e.g., by modifying the vector to comprise the at least one nucleotide sequence.

These methods can include substantially co-temporal expression from: (i) a first nucleotide sequence comprising at least one nucleotide sequence of interest, and (ii) a second nucleotide sequence comprising at least one nucleotide sequence encoding a transcription factor, or at least one nucleotide sequence encoding a translation factor or at least one nucleotide sequence encoding a transcription factor and a translation factor. The vector also can be modified to comprise the at least one nucleotide sequence of interest. The at least one nucleotide sequence of interest can be at least one coding nucleotide sequence. The vector preferably has substantially co-temporal or contemporaneous expression of the first and second nucleotide sequences.

The substantially co-temporal expression can occur by employing promoters for the first and second nucleotide sequences which are functional at approximately the same time or stage of infection. Thus, the nucleotide sequence of interest and the nucleotide sequences encoding the factor(s) can be positioned at different loci in the vector. Alternatively, substantially co-temporal expression can occur by positioning the first and second nucleotide sequences within the same loci. Thus, substantially co-temporal expression can occur by operably linking to the nucleotide sequence of interest and/or to a promoter

operably linked to the nucleotide sequence of interest, a nucleotide sequence encoding a transcription factor, a nucleotide sequence encoding a translation factor, or a nucleotide sequence encoding a transcription factor and a
5 translation factor.

The transcription factor can be from any suitable system. Preferably, the transcription factor is of poxvirus origin, e.g., from a vaccinia virus. The transcription factor can be from expression from an open
10 reading frame selected from the group consisting of H4L, D6, A7, G8R, A1L, A2L, H5R, a homolog thereof and combinations thereof. It is also preferred that embodiments including a nucleotide sequence encoding a transcription factor comprise a poxvirus vector system.
15

The translation factor can likewise be from any suitable system. Preferably the translation factor can effect inhibition of eIF-2 α phosphorylation or inhibition of PKR phosphorylation or otherwise decreases cellular dsRNA content which increases the effective concentration
20 of dsRNA. The translation factor can be selected from expression from the group consisting of: a K3L open reading frame, an E3L open reading frame, a VAI RNA, an EBER RNA, a sigma 3 open reading frame, a TRBP open reading frame, a homolog thereof, and combinations thereof.
25 The term "effective" with respect to dsRNA concentration means the amount of dsRNA to activate PKR and/or eIF-2 α phosphorylation (the dsRNA being in a form therefor). With respect to RNA-based factors, e.g., VAI RNA, EBER RNA, the skilled artisan can obtain suitable
30 DNA from the RNA for use in a DNA vector system without undue experimentation. And, with respect to DNA-based factors, the skilled artisan can obtain suitable RNA therefrom for use in a RNA vector system, without undue experimentation.

35 The term "substantially co-temporal expression" or the term "substantially contemporaneous expression" means that the nucleotide sequence(s) encoding the

transcription or translation or transcription and translation factor(s) are expressed during approximately the same stage of infection as is the at least one nucleotide sequence of interest.

5 For instance, poxvirus genes are regulated in a temporal manner (Coupar, et al., Eur. J. Immunol., 1986, 16:1479-1487, at 1479). Thus, immediately after infection, a class of "early" genes is expressed (Id.). "Early genes" cease being expressed (i.e., early
10 promoters cease functioning) at a time after infection prior to the "later" stage of infection (DNA replication commencement). The thymidine kinase ("TK") gene and TK promoter is an example of an immediate "early" gene and promoter (Hruby et al., J. Virol., 1982, 43(2):403-409, 15 at 403). The TK gene is switched "off" about four hours after infection. "Late genes" are a class of genes not expressed until DNA replication has commenced (Coupar et al., *supra*). The PL11 promoter employed by Coupar et al. is an example of a "late" promoter. Thus,
20 in Coupar et al., HA gene expression regulated by the PL11 promoter was not until after DNA replication, despite being in the TK region.

In contrast to canonical "early" genes and "late" genes the 7.5 kD gene and 7.5 kD promoter, is an
25 example of an "early and late" gene and promoter. An "apparent exception to regulated transcription" (Davison and Moss, "Structure of Vaccinia Virus Early Promoters" J. Mol. Biol., 210-69, 249-69 (1989) at 749), the 7.5 kD promoter "contains regulatory signal for both early and
30 late transcription" (Coupar et al., *supra*). Indeed, there are "independent early and late RNA start sites within the promoter region of the 7.5-kD gene" (Cochran et al., J. Virol., 59(1): 30-37 (April, 1985)).

Coupar et al. observed "that temporal
35 regulation of HA expression by the promoters PF [early], P7.5 [early and late] and PL11 [late] was maintained when the promoters were transposed to interrupt the TK gene of

[vaccinia virus]" (Id., at 1482). That is, Coupar et al. observed that foreign gene expression under the control of an early vaccinia promoter occurred "early", foreign gene expression under control of a late vaccinia promoter 5 occurred "late", and foreign gene expression under the control of the early and late vaccinia 7.5 kD promoter occurred both early and late (See also id. at 1479: "[p]romoter sequences transposed to within the thymidine kinase (TK) gene continue to function in a temporally 10 regulated manner" (citations omitted)).

Thus, the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be under the control of a first type of promoter and the at least one nucleotide sequence 15 of interest or the coding nucleotide sequence can be under the control of a second type of promoter, wherein the first and second promoters are both early, both late (including intermediate), or both early and late; or, the first promoter can be early or late and the second 20 promoter early and late; or the first promoter can be early and late and the second promoter early or late. The nucleotide sequence of interest and the nucleotide sequence(s) encoding the transcription or translation or transcription and translation factor(s) can be at the 25 same locus or at different loci; or under the control of the same promoter.

Accordingly, the invention can relate to a method for preparing a vector having enhanced transcription or translation or transcription and 30 translation and/or expression, or to a method for increasing or enhancing transcription or translation or transcription and translation and/or expression in a vector comprising operably linking to at least one nucleotide sequence of interest, or to a promoter 35 operably linked thereto, at least one nucleotide sequence for at least one transcription and/or at least one translation factor; e.g., at least one nucleotide

sequence for a transcription transcription factor, or at least one nucleotide sequence for a translation factor or at least one nucleotide sequence for a transcription factor and a translation factor. Preferably the
5 translation factor effects an inhibition of eIF-2 α phosphorylation and/or effects an inhibition of phosphorylation of PKR and/or a cellular kinase responsible for phosphorylation of eIF-2 α and/or effects the effective concentration of dsRNA. The invention also
10 can thus relate to vectors from such methods.

Alternatively, the inventive methods can comprise operably linking at least one nucleotide sequence of interest to a first type of promoter and operably linking at least one second nucleotide sequence
15 encoding at least one transcription and/or translation factor to a second type of promoter, within a vector, wherein the first and second promoters are both functional at the same time or same stage of infection, e.g., the first and second promoters are both early, both
20 late (including intermediate), or both early and late; or, the first promoter is early or late and the second promoter is early and late; or the first promoter is early and late and the second promoter is early or late. Of course, the first and second promoters can be the same
25 promoter at two or more different loci, or the same promoter at one locus. And, the invention thus relates to vectors from such methods.

And, the term "nucleotide sequence" as used herein can mean nucleic acid molecule. Thus, a
30 nucleotide sequence can be an isolated nucleic acid molecule, e.g., exogenous DNA.

Accordingly, the present invention can provide vectors modified to contain at least one exogenous nucleotide sequence, preferably encoding at least one epitope of interest, and at least one transcription factor or at least one translation factor or at least one transcription factor and at least one translation factor,
35

wherein there is substantially temporal co-expression (or substantially co-temporal expression or substantially contemporaneous expression) of the exogenous nucleotide sequence and the factor(s); and, methods for making and
5 using such vectors and products therefrom. Enhanced or improved expression is obtained by the vectors and methods of the invention; and, enhanced or improved expression can mean enhanced levels and/or persistence of expression.

10 The invention can thus provide vectors, for instance, poxvirus vectors, which are abortive early, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof,
15 e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the levels and/or persistence of an inserted nucleotide sequence, e.g., a
20 foreign gene. The invention can also provide vectors, for instance, poxvirus vectors, which are abortive late (which includes abortive intermediate), e.g., MVA recombinants, having a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -
25 2, -3, -4, P3, VLTF-X) open reading frame (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus, plasmid or naked DNA, and the like) as a means for enhancing and/or increasing the
30 levels and/or persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

The invention can additionally provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having expression from the
35 vaccinia E3L and/or K3L (or a homolog thereof, e.g., from another vector system, such as poxviruses other than vaccinia, herpesvirus, such as Epstein-Barr, adenovirus,

plasmid or naked DNA, and the like, note discussion *supra* of viral mechanisms to overcome the cellular anti-viral response of translational down-regulation through PKR activation) as a means for enhancing and/or increasing 5 the levels and persistence of an inserted nucleotide sequence, e.g., a foreign gene.

Even further still, the invention can provide vectors, for instance, poxvirus vectors, e.g., NYVAC, ALVAC or TROVAC recombinants, having an early expressed 10 transcription factor, e.g., an early expressed H4L open reading frame (or a homolog thereof) and/or a late expressed transcription factor, e.g., an expressed G8R, A1L, A2L, H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) (or a homolog thereof), for instance abortive late (which 15 includes abortive intermediate), e.g., MVA, recombinants, and expression from the vaccinia E3L and/or K3L (or a homolog thereof) as a means for enhancing and/or increasing the levels and persistence of expression from an inserted nucleotide sequence, e.g., a foreign gene.

20 As shown in the Examples below, ALVAC-HIV recombinant vCP1452 containing the K3L/E3L factors had enhanced expression on human cells in comparison to vCP1433 or vCP300. Indeed, enhanced expression is observed with the E3L/K3L translational factors in human 25 and canine cells.

Enhanced expression by translational factors such as E3L/K3L may be cell type dependent. For instance, while enhanced expression with E3L/K3L is 30 observed in human and canine cells it is not observed in murine and feline cells. From this disclosure and the knowledge in the art, the skilled artisan can select an appropriate translational factor for use with a particular cell type, without undue experimentation. For example, it should go without saying that the skilled 35 artisan knows the differences between cells. Thus it is preferred that the translational factor be expressed in a cell in which enhanced expression is observed, e.g., that

the translational factor employed be with respect to the cell.

Further, preliminary immunogenicity studies in mice show no evidence of enhanced immunogenicity by the 5 E3L/K3L translational factor. This corresponds to no observed enhanced expression in murine cells.

Accordingly, the skilled artisan from this disclosure and the knowledge in the art can select a translational factor which will provide enhanced immunogenicity in a 10 desired animal, without undue experimentation. If enhanced expression is observed *in vitro* in a particular cell line by a particular translational factor, e.g., E3L/K3L in human or canine cells, the skilled artisan can thus expect enhanced immunogenicity *in vivo* in the animal 15 (including human) from which the cells were derived by that particular translational factor, e.g., enhanced immunogenicity in humans and canines from the E3L/K3L translational factor.

Furthermore, in murine cells, the limiting 20 factor of ALVAC expression is at the transcription level. Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be a factor in *in vitro* or *in vivo* applications of the 25 invention (note H4 data), as may be the nature of the vector, e.g., the phenotype of the vector; but, appropriate selection of a cell and vector phenotype and of time of expression from factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled 30 artisan, from this disclosure and the knowledge in the art, without undue experimentation.

Also, the Examples below show that NYVAC recombinant vP1380 has enhanced expression levels in comparison to vP994. Possibly, part of the enhanced 35 levels in vP1380 are due to enhanced transcription and expression from viral specific products such as E3L, such that there is enhanced transcription and translation

involved in expression in vP1380. There is more expression from the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and
5 more persistent levels of expression.

Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994. Another observation is that enhancement profiles are seen
10 in restrictive early cells in the abortive early NYVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication, e.g., VERO or CEF, suggesting that it may be preferred that the factor and the foreign DNA be expressed
15 substantially co-temporally or contemporaneously, i.e., that preferably there be co-expression at substantially the same time or stage, and that the time of expression, e.g., early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early,
20 abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an
25 abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and the transcriptional or translational or transcriptional and translational factor(s) early; in an abortive late system, one preferably expresses exogenous DNA and the
30 transcriptional or translational or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).
35

The methods for making a vector or recombinant can be by or analogous to the methods disclosed in U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941,

5,338,683, 5,494,807, and 4,722,848, WO 95/30018,
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al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also U.S. applications Serial Nos. 08/675,566 and 08/675,556, relating to vectors, including adenovirus vectors.

As to the inserted nucleic acid molecule in a vector of the invention, e.g., the foreign gene, the heterologous or exogenous nucleic acid molecule, e.g., DNA, in vectors of the instant invention, preferably encodes an expression product comprising: an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene or a fusion protein. With respect to these terms, reference is made to the following discussion, and generally to Kendrew, THE ENCYCLOPEDIA OF MOLECULAR BIOLOGY (Blackwell Science Ltd., 1995) and Sambrook, Fritsch and Maniatis, Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1982.

An epitope of interest is an immunologically relevant region of an antigen or immunogen or immunologically active fragment thereof, e.g., from a pathogen or toxin of veterinary or human interest.

An epitope of interest can be prepared from an antigen of a pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a canine distemper virus or measles or rinderpest antigen such as HA or F; a rabies glycoprotein, e.g., rabies glycoprotein G; an avian influenza antigen, e.g., turkey influenza HA, Chicken/Pennsylvania/1/83 influenza antigen such as a nucleoprotein (NP); a bovine leukemia virus antigen, e.g.,

gp51,30 envelope; a Newcastle Disease Virus (NDV) antigen, e.g., HN or F; a feline leukemia virus antigen (FeLV), e.g., FeLV envelope protein; RAV-1 env; matrix and/or preplomer of infectious bronchitis virus; a

5 Herpesvirus glycoprotein, e.g., a glycoprotein from feline herpesvirus, equine herpesvirus, bovine herpesvirus, pseudorabies virus, canine herpesvirus, HSV, Marek's Disease Virus, or cytomegalovirus; a flavivirus antigen, e.g., a Japanese encephalitis virus (JEV)

10 antigen, a Yellow Fever antigen, or a Dengue virus antigen; a malaria (*Plasmodium*) antigen, an immunodeficiency virus antigen, e.g., a feline immunodeficiency virus (FIV) antigen or a simian immunodeficiency virus (SIV) antigen or a human

15 immunodeficiency virus antigen (HIV); a parvovirus antigen, e.g., canine parvovirus; an equine influenza antigen; an poxvirus antigen, e.g., an ectromelia antigen, a canarypox virus antigen or a fowlpox virus antigen; or an infectious bursal disease virus antigen,

20 e.g., VP2, VP3, VP4.

An epitope of interest can be from an antigen of a human pathogen or toxin, or from another antigen or toxin which elicits a response with respect to the pathogen, or from another antigen or toxin which elicits

25 a response with respect to the pathogen, such as, for instance: a Morbillivirus antigen, e.g., a measles virus antigen such as HA or F; a rabies glycoprotein, e.g., rabies virus glycoprotein G; an influenza antigen, e.g., influenza virus HA or N; a Herpesvirus antigen, e.g., a

30 glycoprotein of a herpes simplex virus (HSV), a human cytomegalovirus (HCMV), Epstein-Barr; a flavivirus antigen, a JEV, Yellow Fever virus or Dengue virus antigen; a Hepatitis virus antigen, e.g., HBsAg; an immunodeficiency virus antigen, e.g., an HIV antigen such

35 as gp120, gp160; a Hantaan virus antigen; a C. tetani antigen; a mumps antigen; a pneumococcal antigen, e.g., PspA; a *Borrelia* antigen, e.g., OspA, OspB, OspC of

Borrelia associated with Lyme disease such as *Borrelia burgdorferi*, *Borrelia afzelli* and *Borrelia garinii*; a chicken pox (varicella zoster) antigen; or a *Plasmodium* antigen.

5 Of course, the foregoing lists are intended as exemplary, as the epitope of interest can be derived from any antigen of any veterinary or human pathogen; and, to obtain an epitope of interest, one can express an antigen of any veterinary or human pathogen (such that the
10 invention encompasses the exogenous or foreign nucleic acid molecule(s) of interest encoding at least one antigen).

Since the heterologous DNA can be a growth factor or therapeutic gene, the inventive recombinants
15 can be used in gene therapy. Gene therapy involves transferring genetic information; and, with respect to gene therapy and immunotherapy, reference is made to U.S. Patent No. 5,252,479, which is incorporated herein by reference, together with the documents cited in it and on
20 its face, and to WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also incorporated herein by reference, together with the documents cited therein. The growth factor or therapeutic gene, for example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin, macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7.

The invention further relates to an immunogenic, immunological or vaccine composition

containing the inventive vector and an acceptable carrier or diluent (e.g., veterinary acceptable or pharmaceutically acceptable). An immunological composition containing the vector (or an expression product thereof) elicits an immunological response - local or systemic. The response can, but need not be protective. An immunogenic composition containing the inventive recombinants (or an expression product thereof) likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The invention therefore also provides a method for inducing an immunological response in a host vertebrate comprising administering to the host an immunogenic, immunological or vaccine composition comprising the inventive recombinant virus or vector and an acceptable carrier or diluent. For purposes of this specification, "animal" includes all vertebrate species, except humans; and "vertebrate" includes all vertebrates, including animals (as "animal" is used herein) and humans. And, of course, a subset of "animal" is "mammal", which for purposes of this specification includes all mammals, except humans.

For human administration, the inventive recombinants or vectors, can provide the advantage of expression without productive replication. This thus provides the ability to use recombinants of the invention in immunocompromised individuals; and, provides a level of safety to workers in contact with recombinants of the invention. Therefore, the invention comprehends methods for amplifying or expressing a protein by administering or inoculating a host with a recombinant virus or vector, whereby the host is not a natural host of the recombinant

virus or vector, and there is expression without productive replication.

The exogenous or heterologous DNA (or DNA foreign to vaccine virus) can be DNA encoding any of the 5 aforementioned epitopes of interest, as listed above. In this regard, with respect to *Borrelia* DNA, reference is made to U.S. Patent No. 5,523,089, WO93/08306, PCT/US92/08697, Molecular Microbiology (1989), 3(4), 479-486, and PCT publications WO 93/04175, and WO 96/06165, 10 incorporated herein by reference.

With respect to pneumococcal epitopes of interest, reference is made to Briles et al. WO 92/14488, incorporated herein by reference, with respect to tumor viruses reference is made to Molecular Biology of Tumor 15 Viruses, RNA TUMOR VIRUSES (Second Edition, Edited by Weiss et al., Cold Spring Harbor Laboratory 1982) (e.g., page 44 et seq. - Taxonomy of Retroviruses), incorporated herein by reference.

With respect to DNA encoding epitopes of 20 interest, attention is directed to documents cited herein, see, e.g., documents cited *supra* and documents cited *infra*, for instance: U.S. Patents Nos. 5,174,993 and 5,505,941 (e.g., recombinant avipox virus, vaccinia virus; rabies glycoprotein (G), gene, turkey influenza 25 hemagglutinin gene, gp51,30 envelope gene of bovine leukemia virus, Newcastle Disease Virus (NDV) antigen, FelV envelope gene, RAV-1 env gene, NP (nudeoprotein gene of Chicken/Pennsylvania/1/83 influenza virus), matrix and preplomer gene of infectious bronchitis virus; HSV gD), 30 U.S. Patent No. 5,338,683 (e.g., recombinant vaccinia virus, avipox virus; DNA encoding Herpesvirus glycoproteins, *inter alia*), U.S. Patent No. 5,494,807 (e.g., recombinant vaccinia, avipox; exogenous DNA encoding antigens from rabies, Hepatitis B, JEV, YF, 35 Dengue, measles, pseudorabies, Epstein-Barr, HSV, HIV, SIV, EHV, BHV, HCMV, canine parvovirus, equine influenza, FeLV, FHV, Hantaan, *C. tetani*, avian influenza, mumps,

NDV, *inter alia*), U.S. Patent No. 5,503,834 (e.g., recombinant vaccinia, avipox, Morbillivirus, e.g., measles F, hemagglutinin, *inter alia*), U.S. Patent No. 4,722,848 (e.g., recombinant vaccinia virus; HSV tk, HSV 5 glycoproteins, e.g., gB, gD, influenza HA, Hepatitis B, e.g., HBsAg, *inter alia*), U.K. Patent GB 2 269 820 B and U.S. Patent No. 5,514,375 (recombinant poxvirus; flavivirus structural proteins); WO 92/22641 and U.S. applications Serial Nos. 08/417,210 and 08/372,664 (e.g., 10 recombinant poxvirus; immunodeficiency virus, HTLV, *inter alia*), WO 93/03145 and allowed U.S. applications 08/204,729 and 08/303,124 (e.g., recombinant poxvirus; IBDV, *inter alia*), WO 94/16716 and allowed U.S. 15 application Serial No. 08/184,009, filed January 19, 1994 (e.g., recombinant poxvirus; cytokine and/or tumor associated antigens, *inter alia*), U.S. application Serial No. 08/469,969 (rabies combination compositions), U.S. application Serial No. 08/746,668 (lentivirus, retrovirus and/or immunodeficiency virus, including feline 20 immunodeficiency virus, *inter alia*), U.S. Patent No. 5,529,780 and allowed U.S. application Serial No. 08/413,118 (canine herpesvirus), U.S. application Serial No. 08/471,025 (calicivirus), WO 96/3941 and U.S. 25 application Serial No. 08/658,665 (cytomegalovirus), and PCT/US94/06652 (*Plasmodium* antigens such as from each stage of the *Plasmodium* life cycle).

As to antigens for use in vaccine or immunological compositions, reference is made to the documents and discussion set forth in the documents cited 30 herein (see, e.g., documents cited *supra*); see also Stedman's Medical Dictionary (24th edition, 1982, e.g., definition of vaccine (for a list of antigens used in vaccine formulations; such antigens or epitopes of interest from those antigens can be used in the 35 invention, as either an expression product of an inventive recombinant virus or vector, or in a multivalent composition containing an inventive

recombinant virus or vector or an expression product therefrom).

As to epitopes of interest, one skilled in the art can determine an epitope or immunodominant region of 5 a peptide or polypeptide and ergo the coding DNA therefor from the knowledge of the amino acid and corresponding DNA sequences of the peptide or polypeptide, as well as from the nature of particular amino acids (e.g., size, charge, etc.) and the codon dictionary, without undue 10 experimentation.

A general method for determining which portions of a protein to use in an immunological composition focuses on the size and sequence of the antigen of interest. "In general, large proteins, because they have 15 more potential determinants are better antigens than small ones. The more foreign an antigen, that is the less similar to self configurations which induce tolerance, the more effective it is in provoking an immune response." Ivan Roitt, Essential Immunology, 1988.

20 As to size: the skilled artisan can maximize the size of the protein encoded by the DNA sequence to be inserted into the viral vector (keeping in mind the packaging limitations of the vector). To minimize the DNA inserted while maximizing the size of the protein 25 expressed, the DNA sequence can exclude introns (regions of a gene which are transcribed but which are subsequently excised from the primary RNA transcript).

At a minimum, the DNA sequence can code for a peptide at least 8 or 9 amino acids long. This is the 30 minimum length that a peptide needs to be in order to stimulate a CD8+ T cell response (which recognizes virus infected cells or cancerous cells). A minimum peptide length of 13 to 25 amino acids is useful to stimulate a CD4+ T cell response (which recognizes special antigen 35 presenting cells which have engulfed the pathogen). See Kendrew, *supra*. However, as these are minimum lengths, these peptides are likely to generate an immunological

response, i.e., an antibody or T cell response; but, for a protective response (as from a vaccine composition), a longer peptide is preferred.

With respect to the sequence, the DNA sequence 5 preferably encodes at least regions of the peptide that generate an antibody response or a T cell response. One method to determine T and B cell epitopes involves epitope mapping. The protein of interest "is fragmented into overlapping peptides with proteolytic enzymes. The 10 individual peptides are then tested for their ability to bind to an antibody elicited by the native protein or to induce T cell or B cell activation. This approach has been particularly useful in mapping T-cell epitopes since the T cell recognizes short linear peptides complexed 15 with MHC molecules. The method is less effective for determining B-cell epitopes" since B cell epitopes are often not linear amino acid sequence but rather result from the tertiary structure of the folded three dimensional protein. Janis Kuby, Immunology, (1992) pp. 20 79-80.

Another method for determining an epitope of interest is to choose the regions of the protein that are hydrophilic. Hydrophilic residues are often on the surface of the protein and are therefore often the 25 regions of the protein which are accessible to the antibody. Janis Kuby, Immunology, (1992) p. 81

Yet another method for determining an epitope of interest is to perform an X-ray crystallographic analysis of the antigen (full length)-antibody complex. 30 Janis Kuby, Immunology, (1992) p. 80.

Still another method for choosing an epitope of interest which can generate a T cell response is to identify from the protein sequence potential HLA anchor binding motifs which are peptide sequences which are 35 known to be likely to bind to the MHC molecule.

The peptide which is a putative epitope of interest, to generate a T cell response, should be

presented in a MHC complex. The peptide preferably contains appropriate anchor motifs for binding to the MHC molecules, and should bind with high enough affinity to generate an immune response. Factors which can be
5 considered are: the HLA type of the patient (vertebrate, animal or human) expected to be immunized, the sequence of the protein, the presence of appropriate anchor motifs and the occurrence of the peptide sequence in other vital cells.

10 An immune response is generated, in general, as follows: T cells recognize proteins only when the protein has been cleaved into smaller peptides and is presented in a complex called the "major histocompatibility complex MHC" located on another cell's surface. There are two
15 classes of MHC complexes - class I and class II, and each class is made up of many different alleles. Different patients have different types of MHC complex alleles; they are said to have a 'different HLA type.'

Class I MHC complexes are found on virtually
20 every cell and present peptides from proteins produced inside the cell. Thus, Class I MHC complexes are useful for killing cells which when infected by viruses or which have become cancerous and as the result of expression of an oncogene. T cells which have a protein called CD8 on
25 their surface, bind specifically to the MHC class I/peptide complexes via the T cell receptor. This leads to cytolytic effector activities.

Class II MHC complexes are found only on antigen-presenting cells and are used to present
30 peptides from circulating pathogens which have been endocytosed by the antigen-presenting cells. T cells which have a protein called CD4 bind to the MHC class II/peptide complexes via the T cell receptor. This leads to the synthesis of specific cytokines which stimulate an
35 immune response.

Some guidelines in determining whether a protein is an epitope of interest which will stimulate a

T cell response, include: Peptide length - the peptide should be at least 8 or 9 amino acids long to fit into the MHC class I complex and at least 13-25 amino acids long to fit into a class II MHC complex. This length is 5 a minimum for the peptide to bind to the MHC complex. It is preferred for the peptides to be longer than these lengths because cells may cut the expressed peptides. The peptide should contain an appropriate anchor motif which will enable it to bind to the various class I or 10 class II molecules with high enough specificity to generate an immune response (See Bocchia, M. et al, Specific Binding of Leukemia Oncogene Fusion Protein Peptides to HLA Class I Molecules, Blood 85:2680-2684; Englehard, VH, Structure of peptides associated with 15 class I and class II MHC molecules Ann. Rev. Immunol. 12:181 (1994)). This can be done, without undue experimentation, by comparing the sequence of the protein of interest with published structures of peptides associated with the MHC molecules. Protein epitopes 20 recognized by T cell receptors are peptides generated by enzymatic degradation of the protein molecule and are presented on the cell surface in association with class I or class II MHC molecules.

Further, the skilled artisan can ascertain an 25 epitope of interest by comparing the protein sequence with sequences listed in the protein data base.

Even further, another method is simply to generate or express portions of a protein of interest, generate monoclonal antibodies to those portions of the 30 protein of interest, and then ascertain whether those antibodies inhibit growth *in vitro* of the pathogen from which the from which the protein was derived. The skilled artisan can use the other guidelines set forth in this disclosure and in the art for generating or 35 expressing portions of a protein of interest for analysis as to whether antibodies thereto inhibit growth *in vitro*. For example, the skilled artisan can generate portions of

a protein of interest by: selecting 8 to 9 or 13 to 25 amino acid length portions of the protein, selecting hydrophylic regions, selecting portions shown to bind from X-ray data of the antigen (full length)-antibody complex, selecting regions which differ in sequence from other proteins, selecting potential HLA anchor binding motifs, or any combination of these methods or other methods known in the art.

Epitopes recognized by antibodies are expressed on the surface of a protein. To determine the regions of a protein most likely to stimulate an antibody response one skilled in the art can preferably perform an epitope map, using the general methods described above, or other mapping methods known in the art.

As can be seen from the foregoing, without undue experimentation, from this disclosure and the knowledge in the art, the skilled artisan can ascertain the amino acid and corresponding DNA sequence of an epitope of interest for obtaining a T cell, B cell and/or antibody response. In addition, reference is made to Gefter et al., U.S. Patent No. 5,019,384, issued May 28, 1991, and the documents it cites, incorporated herein by reference (Note especially the "Relevant Literature" section of this patent, and column 13 of this patent which discloses that: "A large number of epitopes have been defined for a wide variety of organisms of interest. Of particular interest are those epitopes to which neutralizing antibodies are directed. Disclosures of such epitopes are in many of the references cited in the Relevant Literature section.")

With respect to expression of a biological response modulator, reference is made to Wohlstadter, "Selection Methods," WO 93/19170, published 30 September 1993, and the documents cited therein, incorporated herein by reference.

For instance, a biological response modulator modulates biological activity; for instance, a biological

response modulator is a modulatory component such as a high molecular weight protein associated with non-NMDA excitatory amino acid receptors and which allosterically regulates affinity of AMPA binding (See Kendrew, *supra*).

5 The recombinant of the present invention can express such a high molecular weight protein.

More generally, nature has provided a number of precedents of biological response modulators. Modulation of activity may be carried out through mechanisms as 10 complicated and intricate as allosteric induced quaternary change to simple presence/absence, e.g., expression/degradation, systems. Indeed, the repression/activation of expression of many biological molecules is itself mediated by molecules whose 15 activities are capable of being modulated through a variety of mechanisms.

Table 2 of Neidhardt et al *Physiology of the Bacterial Cell* (Sinauer Associates Inc., Publishers, 1990), at page 73, lists chemical modifications to 20 bacterial proteins. As is noted in that table, some modifications are involved in proper assembly and other modifications are not, but in either case such modifications are capable of causing modulation of function. From that table, analogous chemical 25 modulations for proteins of other cells can be determined, without undue experimentation.

In some instances modulation of biological functions may be mediated simply through the proper/improper localization of a molecule. Molecules 30 may function to provide a growth advantage or disadvantage only if they are targeted to a particular location. For example, a molecule may be typically not taken up or used by a cell, as a function of that molecule being first degraded by the cell by secretion of 35 an enzyme for that degradation. Thus, production of the enzyme by a recombinant can regulate use or uptake of the molecule by a cell. Likewise, the recombinant can

express a molecule which binds to the enzyme necessary for uptake or use of a molecule, thereby similarly regulating its uptake or use.

Localization targeting of proteins carried out 5 through cleavage of signal peptides another type of modulation or regulation. In this case, a specific endoprotease catalytic activity can be expressed by the recombinant.

Other examples of mechanisms through which 10 modulation of function may occur are RNA virus poly-proteins, allosteric effects, and general covalent and non-covalent steric hindrance. HIV is a well studied example of an RNA virus which expresses non-functional poly-protein constructs. In HIV "the gag, pol, and env 15 poly-proteins are processed to yield, respectively, the viral structural proteins p17, p24, and p15--reverse transcriptase and integrase--and the two envelope proteins gp41 and gp120" (Kohl et al., PNAS USA 85:4686-90 (1988)). The proper cleavage of the poly-proteins is 20 crucial for replication of the virus, and virions carrying inactive mutant HIV protease are non-infectious (*Id.*). This is another example of the fusion of proteins down-modulating their activity. Thus, it is possible to construct recombinant viruses which express molecules 25 which interfere with endoproteases, or which provide endoproteases, for inhibiting or enhancing the natural expression of certain proteins (by interfering with or enhancing cleavage).

The functional usefulness of enzymes may also 30 be modulated by altering their capability of catalyzing a reaction. Illustrative examples of modulated molecules are zymogens, formation/disassociation of multi-subunit functional complexes, RNA virus poly-protein chains, allosteric interactions, general steric hindrance 35 (covalent and non-covalent) and a variety of chemical modifications such as phosphorylation, methylation, acetylation, adenylation, and uridylation (see Table 1

of Neidhardt, *supra*, at page 315 and Table 2 at page 73).

Zymogens are examples of naturally occurring protein fusions which cause modulation of enzymatic activity. Zymogens are one class of proteins which are converted into their active state through limited proteolysis. See Table 3 of Reich, *Proteases and Biological Control, Vol. 2*, (1975) at page 54). Nature has developed a mechanism of down-modulating the activity of certain enzymes, such as trypsin, by expressing these enzymes with additional "leader" peptide sequences at their amino termini. With the extra peptide sequence the enzyme is in the inactive zymogen state. Upon cleavage of this sequence the zymogen is converted to its enzymatically active state. The overall reaction rates of the zymogen are "about 10^5 - 10^6 times lower than those of the corresponding enzyme" (See Table 3 of Reich, *supra* at page 54).

It is therefore possible to down-modulate the function of certain enzymes simply by the addition of a peptide sequence to one of its termini. For example, with knowledge of this property, a recombinant can express peptide sequences containing additional amino acids at one or both termini.

The formation or disassociation of multi-subunit enzymes is another way through which modulation may occur. Different mechanisms may be responsible for the modulation of activity upon formation or disassociation of multi-subunit enzymes.

Therefore, sterically hindering the proper specific subunit interactions will down-modulate the catalytic activity. And accordingly, the recombinant of the invention can express a molecule which sterically hinders a naturally occurring enzyme or enzyme complex, so as to modulate biological functions.

Certain enzyme inhibitors afford good examples of functional down-modulation through covalent steric hindrance or modification. Suicide substrates which

irreversibly bind to the active site of an enzyme at a catalytically important amino acid in the active site are examples of covalent modifications which sterically block the enzymatic active site. An example of a suicide 5 substrate is TPCK for chymotrypsin (*Fritsch, Enzyme Structure and Mechanism*, 2d ed; Freeman & Co. Publishers, 1984)). This type of modulation is possible by the recombinant expressing a suitable suicide substrate, to thereby modulate biological responses (e.g., by limiting 10 enzyme activity).

There are also examples of non-covalent steric hindrance including many repressor molecules. The recombinant can express repressor molecules which are capable of sterically hindering and thus down-modulating 15 the function of a DNA sequence by preventing particular DNA-RNA polymerase interactions.

Allosteric effects are another way through which modulation is carried out in some biological systems. Aspartate transcarbamoylase is a well 20 characterized allosteric enzyme. Interacting with the catalytic subunits are regulatory domains. Upon binding to CTP or UTP the regulatory subunits are capable of inducing a quaternary structural change in the holoenzyme causing down-modulation of catalytic activity. In 25 contrast, binding of ATP to the regulatory subunits is capable of causing up-modulation of catalytic activity (*Fritsch, supra*). Using methods of the invention, molecules can be expressed which are capable of binding and causing modulatory quaternary or tertiary changes.

30 In addition, a variety of chemical modifications, e.g., phosphorylation, methylation, acetylation, adenylation, and uridylation may be carried out so as to modulate function. It is known that modifications such as these play important roles in the 35 regulation of many important cellular components. Table 2 of *Neidhardt, supra*, at page 73, lists different bacterial enzymes which undergo such modifications. From

that list, one skilled in the art can ascertain other enzymes of other systems which undergo the same or similar modifications, without undue experimentation. In addition, many proteins which are implicated in human disease also undergo such chemical modifications. For example, many oncogenes have been found to be modified by phosphorylation or to modify other proteins through phosphorylation or dephosphorylation. Therefore, the ability afforded by the invention to express modulators which can modify or alter function, e.g., phosphorylation, is of importance.

From the foregoing, the skilled artisan can use the present invention to express a biological response modulator, without any undue experimentation.

With respect to expression of fusion proteins by inventive recombinants, reference is made to Sambrook, Fritsch, Maniatis, *Molecular Cloning, A LABORATORY MANUAL* (2d Edition, Cold Spring Harbor Laboratory Press, 1989) (especially Volume 3), and Kendrew, *supra*, incorporated herein by reference. The teachings of Sambrook et al., can be suitably modified, without undue experimentation, from this disclosure, for the skilled artisan to generate recombinants or vectors expressing fusion proteins.

With regard to gene therapy and immunotherapy, reference is made to U.S. Patent Nos. 4,690,915 and 5,252,479, which are incorporated herein by reference, together with the documents cited therein it and on their face, and to WO 94/16716 and U.S. application Serial No. 08/184,009, filed January 19, 1994, each of which is also incorporated herein by reference, together with the documents cited therein.

A growth factor can be defined as multifunctional, locally acting intercellular signalling peptides which control both ontogeny and maintenance of tissue and function (see Kendrew, *supra*, especially at page 455 *et seq.*).

The growth factor or therapeutic gene, for

example, can encode a disease-fighting protein, a molecule for treating cancer, a tumor suppressor, a cytokine, a tumor associated antigen, or interferon; and, the growth factor or therapeutic gene can, for example, 5 be selected from the group consisting of a gene encoding alpha-globin, beta-globin, gamma-globin, granulocyte macrophage-colony stimulating factor, tumor necrosis factor, an interleukin (e.g., an interleukin selected from interleukins 1 to 14, or 1 to 11, or any combination 10 thereof), macrophage colony stimulating factor, granulocyte colony stimulating factor, erythropoietin, mast cell growth factor, tumor suppressor p53, retinoblastoma, interferon, melanoma associated antigen or B7. U.S. Patent No. 5,252,479 provides a list of 15 proteins which can be expressed in an adenovirus system for gene therapy, and the skilled artisan is directed to that disclosure. WO 94/16716 and allowed U.S. application Serial No. 08/184,009, filed January 19, 1994, provide genes for cytokines and tumor associated 20 antigens and immunotherapy methods, including *ex vivo* methods, and the skilled artisan is directed to those disclosures.

Thus, one skilled in the art can create recombinants or vectors expressing a growth factor or 25 therapeutic gene and use the recombinants or vectors, from this disclosure and the knowledge in the art, without undue experimentation.

Moreover, from the foregoing and the knowledge in the art, no undue experimentation is required for the 30 skilled artisan to construct an inventive recombinant or vector which expresses an epitope of interest, a biological response modulator, a growth factor, a recognition sequence, a therapeutic gene, or a fusion protein; or for the skilled artisan to use such a 35 recombinant or vector.

As the recombinants or vectors of the invention can be used for expression of gene products *in vitro*,

techniques for protein purification can be employed in the practice of the invention, and such techniques, in general, include:

Briefly, the cells are disrupted and the 5 protein of interest is released into an aqueous "extract". There are many methods of cellular disintegration, which vary from relatively gentle to vigorous conditions, and the choice of one method over the other is dependent upon the source material. Animal 10 tissues vary from the very easily broken erythrocytes to tough collagenous material such as found in blood vessels and other smooth-muscle containing tissue. Bacteria vary from fairly fragile organisms that can be broken up by digestive enzymes or osmotic shock to more resilient 15 species with thick cell walls, needing vigorous mechanical treatment for disintegration.

Gentle techniques include cell lysis, enzymatic digestion, chemical solubilization, hand homogenization and mincing (or grinding); moderate techniques of cell 20 disintegration include blade homogenization and grinding with abrasive materials, i.e., sand or alumina; and vigorous techniques include french press, ultrasonication, bead mill or Manton-Gaulin homogenization. Each of the aforementioned techniques 25 are art-recognized, and it is well within the scope of knowledge of the skilled artisan to determine the appropriate method for cell disintegration based upon the starting material, and the teachings herein and in the art.

Following cell disintegration, the extract is 30 prepared by centrifuging off insoluble material. At this stage, one may proceed with the purification method, as an extract containing as much of the protein of interest as possible has been prepared, and, where appropriate, 35 particulate and most nonprotein materials have been removed.

Standard techniques of protein purification may

be employed to further purify the protein of interest, including: precipitation by taking advantage of the solubility of the protein of interest at varying salt concentrations, precipitation with organic solvents, 5 polymers and other materials, affinity precipitation and selective denaturation; column chromatography, including high performance liquid chromatography (HPLC), ion-exchange, affinity, immuno affinity or dye-ligand chromatography; immunoprecipitation and the use of gel 10 filtration, electrophoretic methods, ultrafiltration and isoelectric focusing. Each of the above-identified methods are well within the knowledge of the skilled artisan, and no undue experimentation is required to purify the proteins or epitopes of interest from 15 expression of a recombinant or vector of the invention, using the standard methodologies outlined hereinabove, and in the literature, as well as the teachings in the Examples below.

As the expression products can provide an 20 antigenic, immunological, or protective (vaccine) response, the invention further relates to products therefrom; namely, antibodies and uses thereof. More in particular, the expression products can elicit antibodies by administration of those products or of recombinants or 25 vectors expressing the products. The antibodies can be monoclonal antibodies; and, the antibodies or expression products can be used in kits, assays, tests, and the like involving binding, so that the invention relates to these uses too. Additionally, since the recombinants or 30 vectors of the invention can be used to replicate DNA, the invention relates to the inventive recombinants as vectors and methods for replicating DNA by infecting or transfecting cells with the recombinant and harvesting DNA therefrom. The resultant DNA can be used as probes 35 or primers or for amplification.

The administration procedure for the inventive recombinants or vectors or expression products thereof,

compositions of the invention such as immunological, antigenic or vaccine compositions or therapeutic compositions can be via a parenteral route (intradermal, intramuscular or subcutaneous). Such an administration 5 enables a systemic immune response. The administration can be via a mucosal route, e.g., oral, nasal, genital, etc. Such an administration enables a local immune response.

More generally, the inventive antigenic, 10 immunological or vaccine compositions or therapeutic compositions can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical, medical or veterinary arts. Such compositions can be administered in dosages and by 15 techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the breed or species, age, sex, weight, and condition of the particular patient, and the route of administration. The compositions can be administered alone, or can be co- 20 administered or sequentially administered with other compositions of the invention or with other immunological, antigenic or vaccine or therapeutic compositions. Such other compositions can include purified native antigens or epitopes or antigens or 25 epitopes from expression by an inventive recombinant or vector or another vector system; and are administered taking into account the aforementioned factors.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, 30 nasal, anal, genital, e.g., vaginal, etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. 35 In such compositions the recombinant or vector may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or

the like.

Antigenic, immunological or vaccine compositions typically can contain an adjuvant and an amount of the recombinant or vector or expression product to elicit the desired response. In human applications, alum (aluminum phosphate or aluminum hydroxide) is a typical adjuvant. Saponin and its purified component Quil A, Freund's complete adjuvant and other adjuvants used in research and veterinary applications have toxicities which limit their potential use in human vaccines. Chemically defined preparations such as muramyl dipeptide, monophosphoryl lipid A, phospholipid conjugates such as those described by Goodman-Snitkoff et al. J. Immunol. 147:410-415 (1991) and incorporated by reference herein, encapsulation of the protein within a proteoliposome as described by Miller et al., J. Exp. Med. 176:1739-1744 (1992) and incorporated by reference herein, and encapsulation of the protein in lipid vesicles such as Novasome™ lipid vesicles (Micro Vascular Systems, Inc., Nashua, NH) can also be used.

The composition may be packaged in a single dosage form for immunization by parenteral (i.e., intramuscular, intradermal or subcutaneous) administration or orifice administration, e.g., perlingual (i.e., oral), intragastric, mucosal including intraoral, intraanal, intravaginal, and the like administration. And again, the effective dosage and route of administration are determined by the nature of the composition, by the nature of the expression product, by expression level if the recombinant is directly used, and by known factors, such as breed or species, age, sex, weight, condition and nature of host, as well as LD₅₀ and other screening procedures which are known and do not require undue experimentation. Dosages of expressed product can range from a few to a few hundred micrograms, e.g., 5 to 500 µg. The inventive recombinant or vector can be administered in any suitable amount to achieve

expression at these dosage levels. The viral recombinants of the invention can be administered in an amount of about $10^{3.5}$ pfu; thus, the inventive viral recombinant is preferably administered in at least this amount; more preferably about 10^4 pfu to about 10^6 pfu; however higher dosages such as about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, for instance about 10^6 pfu to about 10^8 pfu can be employed. Suitable quantities of inventive plasmid or naked DNA in plasmid or naked DNA compositions can be 1 ug to 100 mg, preferably 0.1 to 10 mg, but lower levels such as 0.1 to 2 mg or preferably 1-10 ug may be employed. Other suitable carriers or diluents can be water or a buffered saline, with or without a preservative. The expression product or recombinant or vector may be lyophilized for resuspension at the time of administration or can be in solution.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nanoparticles, reported by Kreuter, J., Microcapsules and Nanoparticles in Medicine and Pharmacology, M. Donbrow (Ed). CRC Press, p. 125-148.

Microencapsulation has been applied to the injection of microencapsulated pharmaceuticals to give a controlled release. A number of factors contribute to the selection of a particular polymer for microencapsulation. The reproducibility of polymer synthesis and the microencapsulation process, the cost of the microencapsulation materials and process, the toxicological profile, the requirements for variable release kinetics and the physicochemical compatibility of the polymer and the antigens are all factors that must be considered. Examples of useful polymers are

polycarbonates, polyesters, polyurethanes, polyorthoesters and polyamides, particularly those that are biodegradable.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,L-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals including peptides and antigens have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for the controlled release of antigen, for example, as reviewed by Eldridge, J.H., et al.

15 Current Topics in Microbiology and Immunology, 1989, 146:59-66. The entrapment of antigens in PLGA microspheres of 1 to 10 microns in diameter has been shown to have a remarkable adjuvant effect when administered orally. The PLGA microencapsulation process

20 uses a phase separation of a water-in-oil emulsion. The compound of interest is prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed

25 stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of an assortment of agents (polyvinyl alcohol (PVA),

30 gelatin, alginates, polyvinylpyrrolidone (PVP), methyl cellulose) and the solvent removed by either drying *in vacuo* or solvent extraction.

Thus, solid, including solid-containing-liquid, liquid, and gel (including "gel caps") compositions are envisioned.

Furthermore, the inventive vectors or recombinants can be used in any desired immunization or

administration regimen; e.g., as part of periodic vaccinations such as annual vaccinations as in the veterinary arts or as in periodic vaccinations as in the human medical arts, or as in a prime-boost regimen
5 wherein an inventive vector or recombinant is administered either before or after the administration of the same or of a different epitope of interest or recombinant or vector expressing such a same or different epitope of interest (including an inventive recombinant
10 or vector expressing such a same or different epitope of interest), see, e.g., documents cited herein such as U.S. application Serial No. 08/746,668.

Additionally, the inventive vectors or recombinants and the expression products therefrom can
15 stimulate an immune or antibody response in animals. From those antibodies, by techniques well-known in the art, monoclonal antibodies can be prepared and, those monoclonal antibodies, can be employed in well known antibody binding assays, diagnostic kits or tests to
20 determine the presence or absence of antigen(s) and therefrom the presence or absence of the natural causative agent of the antigen or, to determine whether an immune response to that agent or to the antigen(s) has simply been stimulated.

25 Monoclonal antibodies are immunoglobulin produced by hybridoma cells. A monoclonal antibody reacts with a single antigenic determinant and provides greater specificity than a conventional, serum-derived antibody. Furthermore, screening a large number of
30 monoclonal antibodies makes it possible to select an individual antibody with desired specificity, avidity and isotype. Hybridoma cell lines provide a constant, inexpensive source of chemically identical antibodies and preparations of such antibodies can be easily
35 standardized. Methods for producing monoclonal antibodies are well known to those of ordinary skill in the art, e.g., Koprowski, H. et al., U.S. Pat. No.

4,196,265, issued Apr. 1, 1989, incorporated herein by reference.

Uses of monoclonal antibodies are known. One such use is in diagnostic methods, e.g., David, G. and 5 Greene, H., U.S. Pat. No. 4,376,110, issued Mar. 8, 1983, incorporated herein by reference.

Monoclonal antibodies have also been used to recover materials by immunoabsorption chromatography, e.g. Milstein, C., 1980, Scientific American 243:66, 70, 10 incorporated herein by reference.

Furthermore, the inventive recombinants or vectors or expression products therefrom can be used to stimulate a response in cells *in vitro* or *ex vivo* for subsequent reinfusion into a patient. If the patient is 15 seronegative, the reinfusion is to stimulate an immune response, e.g., an immunological or antigenic response such as active immunization. In a seropositive individual, the reinfusion is to stimulate or boost the immune system against a pathogen.

20 The recombinants or vectors of the invention are also useful for generating DNA for probes or for PCR primers which can be used to detect the presence or absence of hybridizable DNA or to amplify DNA, e.g., to detect a pathogen in a sample or for amplifying DNA.

25 Since viruses require translation of viral mRNAs in order to generate viral proteins required for replication, it is evident that any function which blocks the action of PKR in the infected cell will have a positive effect on viral protein expression. Thus, co- 30 expression, in some fashion, of the vaccinia E3L/K3L gene products, or a homolog of E3L and/or K3L, may provide a general mechanism for enhancing the expression levels of heterologous gene products by vectors in general. The E3L/K3L or homologous functions may enhance or augment 35 native anti-PKR mechanisms, and thus increase protein expression levels and/or persistence. This provides a useful element towards optimizing the efficiency of

eukaryotic virus systems as immunization vehicles. This approach could be further extended for improvement of DNA-based immunogens, e.g., naked DNA or plasmid DNA vector systems. Further, employing a nucleotide sequence 5 for a transcription factor, e.g., for an early and/or late viral transcription factor, in conjunction with enhancing translation by employing a nucleotide sequence for a translation factor, can even further enhance or increase expression by increasing or enhancing 10 transcription and translation; and thus, increasing or enhancing levels or persistence of expression can be obtained.

A better understanding of the present invention and of its many advantages will be had from the following 15 non-limiting Examples, given by way of illustration.

EXAMPLES

Example 1 - NYVAC Recombinants Containing H4L

The plasmids placZH6H4L and placZH6H4Lreverse (ATCC Deposit No. ____) were used as donor plasmids for 20 *in vivo* recombination with the rescue virus vP994 (ATCC Deposit No. ____ ; U.S. Patent No. 5,494,807, incorporated herein by reference; vaccinia H6 promoter/HIV1 MN env-noncleavable, secreted gp140, in HA insertion site). The donor plasmids were designed to 25 replace the endogenous promoter and coding sequences of H4L by homologous recombination. The resulting recombinant viruses were designated vP1379 and vP1380; vP1379 contains the H6lacZ/H6H4L cassette in a head-to-head configuration; vP1380 contains the H6lacZ/H6H4L 30 cassette in a head-to-tail configuration (SEQ ID NO: ; Fig. 1).

The plasmids were constructed as follows:

H4L Expression Cassette

The H4L open reading frame (orf) as delineated 35 in Goebel et al. 1990 corresponds to positions 94830-92446 in the Copenhagen (vaccine) strain vaccinia virus genomic sequence. pSD404VC contains a clone of the 8.6Kb

HindIII H fragment of Copenhagen vaccinia virus inserted into the pUC vector background. pSD404VC was digested with Pvull to isolate a 3860bp fragment containing the H4L coding sequences and flanking sequences. The 3860bp 5 fragment was inserted into the blunted BamHI site of pBSecogpt (*E.coli* gpt gene (ATCC No. 37145) under the control of Copenhagen B13R promoter in the pBS SK vector (Stratagene La Jolla, CA.)) resulting in plasmid pRW935.

pRW935 was linearized with EcoRI and partially 10 digested with DraI to remove a 970bp fragment containing the 5' end of the H4L coding sequence. Using a series of Polymerase Chain Reactions (PCRs) the H4L coding sequence was reengineered to be under the control of the modified 15 vaccinia H6 promoter (Perkus et al. 1989). Using the plasmid template pRW935 and primer pairs RW500/RW502 and RW501/RW503 in the PCR amplifications, the 5' H4L sequences were regenerated. In addition, the oligonucleotide, RW502, modifies the H4L coding sequences (position 341-348 from the A of the ATG) from TTTTTTTT to 20 TTTTCTTC without altering the predicted amino acid sequence to remove an early transcriptional stop signal (Yuan, L. and Moss, B., 1987). The modified H6 promoter was amplified from the plasmid template pRW936 using oligonucleotides RW504 and RW507. Oligonucleotides RW505 25 and RW506 having complementary sequences were PCR amplified directly. The four PCR reactions were pooled and further amplified using primer pair RW500 and RW505. The resulting PCR fragment was digested with DraI and EcoRI and cloned into DraI and EcoRI digested pRW935 generating pRW939. A PCR introduced error in the 5' end 30 of the coding region of pRW939 was corrected, resulting in plasmid pRW947. Specifically, the PCR error introduced in pRW939 (H4L codon 155 is AAA - correct codon should be GAA) was corrected by replacement of the 35 600 bp pRW939 AflIII-EcoRI fragment with the equivalent fragment from pRW935 to generate pRW939. The oligonucleotide sequences for each of the above-

identified oligonucleotides (RW500 and RW501 to RW507; SEQ ID NOS:) are:

RW500 5' - GAAATAGTTAGCGTCAAC -3'

RW501 5' - TGTCTAATGTGTTGAAGAAAAGATCATACAAGTTATAC -3'

5 RW502 5' - AACTTGTATGATCTTCACACATTAGACATGTATTAC -3'

RW503 5' - TAAGTTGTATCGTAATGGACTCTAAAGAGACTATTC -3'

RW504 5' - AGTCTCTTAGAGTCATTACGATAACAAACTAAC -3'

RW505

5' -

10 CCGACGATTAAACGCCACCGTCAGGGAAAGTTCTAAGAACGCACCGGAAGAGA AGAGA ATTCTCGGGACAATTGGATC -3'

RW506

5' -

GTCTAGCTGGTGCTGAGTTCTACGTGAGTTGATTGCTCTTGCCTCGTG

15 ATCCAATTGTCCCAGATATTCTC -3'

RW507

5' -

GTAGAAACTCAGCACCAAGCTAGACAAGCTTCTTATTCTATACTAAAAAGTGAAAA TAAATAC -3'

20 The plasmid pRW947 was digested with *Xho*I to

generate two fragments. The 7036 bp fragment containing the H6 promoted H4L in the pBSSK vector background was purified and self-ligated, resulting in the plasmid pH6H4L. The plasmid pRW973A, containing a LacZ

25 expression cassette under the control of the vaccinia H6 promoter, was digested with *Hind*III. The 3.3 Kbp

fragment was purified and ligated into the *Hind*III digested pH6H4L, thereby generating pLACZH6H4Lreverse

(H6 promoted LacZ gene and H6 promoted H4L gene in head-

30 to-tail configuration), and placZH6H4L (H6 promoted lacZ gene and H6 promoted H4L gene in a head-to-head configuration).

Example 2 - ALVAC Recombinants

35 pMPC6H6K3E3 (ATCC No. ____) was used as a donor plasmid in *in vivo* recombination (Piccini et al., 1987) with rescuing virus vCP205 (ATCC No. ____; U.S. application Serial No. 08/417,210, incorporated herein by

reference; HIV expression cassette - vaccinia H6 promoter/HIV truncated env MN strain, I3L gag with protease in ALVAC C3 insertion site); and the resulting recombinant virus was designated vCP1431A (vaccinia 5 H6/K3L and E3L cassette in the C6 locus).

pC8H6H4 was used as the donor plasmid in *in vivo* recombination with vCP205 and the resulting recombinant virus designated vCP1435 (HIV cassette at C3 10 locus and the vaccinia H6/H4L expression cassette at C8 locus; H6/H4L expression cassette flanked by ALVAC C8 insertion site sequences 15 (SEQ ID NO:) shown in Fig. 2).

vCP1431A was also used as a rescuing virus in *in vivo* recombination using plasmid pC8H6H4, generating 15 the recombinant designated vCP1437A (HIV cassette at the C3 locus, the H6/K3L and E3L cassette at the C6 locus, and the vaccinia H6/H4L cassette at the C8 locus). With respect to the H6/K3L expression cassette and the 20 vaccinia E3L gene with the endogenous promoter flanked by the ALVAC C6 insertion site sequences reference is made to Fig. 3 (SEQ ID NO:).

pC3H6FHVB (ATCC No. ____; Fig. 5, SEQ ID NO: ; H6 promoted FHV gB ORF with early transcriptional and translational stop signals at both 5' and 3' ends flanked 25 by the left and right arms of the ALVAC C3 locus) was used in *in vivo* recombination with the ALVAC (ATCC No. VR-2547) to generate vCP1459 (H6 promoted FHV gB expression cassette in deorfed C3 insertion locus). With respect to the FHV-1 gB coding region in which the two 30 internal T₅NT motifs have been mutated, see Fig. 4 (SEQ ID NO:).

pC3H6FHVB was used in *in vivo* recombination with vCP1431A to generate vCP1460 (H6 promoted FHV gB expression cassette in the deorfed C3 insertion locus and 35 vaccinia E3L/K3L genes in C6 locus).

pC3H6FHVB was used in *in vivo* recombination with vCP1437 to generate vCP1464 (H6 promoted FHV gB

expression cassette in deorfed C3 insertion locus, vaccinia E3L/K3L genes in C6 locus and H6 promoted vaccinia H4L ORF in C8 locus).

pMPC5H6PN (HIV pol/nef "string of beads" cassette in the ALVAC C5 locus) was used in recombination with vCP205 to obtain vCP1433 (ATCC Deposit No. ____). Thus, recombinant ALVAC-MN120TMGNPst (vCP1433) was generated by insertion of an expression cassette encoding a synthetic polypeptide containing all of the known Pol CTL epitopes (Nixon and McMichael; 1991) and all of the known human Nef CTL epitopes into vCP205 at the insertion site known as C5.

pMPC6H6K3E3 (ATCC Deposit No. ____ ; containing vaccinia H6/K3L expression cassette and vaccinia E3L gene with endogenous promoter flanked by the ALVAC C6 insertion site sequences) was used in recombination with vCP1433 to obtain vCP1452. Figures 6 and 7 show the nucleotide and amino acid sequences of the vCP1433 and vCP1452 inserts. Figure 8 shows the K3L E3L in C6 in vCP1452. vCP1452 contains the HIV type 1 gag and protease genes derived from the IIIB isolate, the gp120 envelope sequences derived from the MN isolate, and sequences encoding a polypeptide encompassing the known human CTL epitopes from HIV-1 Nef and Pol (Nef1 and Nef2 CTL epitopes, and Pol1, Pol2 and Pol3 CTL epitopes). The expressed gp120 moiety is linked to the transmembrane (TM) anchor sequence (28 amino acids) of the envelope glycoprotein. In addition to the HIV coding sequences vCP1452 contains the vaccinia virus E3L and K3L coding sequences inserted into the C6 site. The insertion sites and promoter linkages for this construct are shown in the Table below.

Table: Insertion sites and promoter linkages in vCP1452

	Insert	Insertion Site	Promoter
	HIV1 MN gp120 + TM	C3	H6
5	HIV1 IIIB gag (+ pro)	C3	I3L
	Pol3/Nef C term/Pol2/Nef central/Pol1	C5	H6
	Vaccinia virus E3L	C6	endogenous
	Vaccinia virus K3L	C6	H6

10 vCP300 is an ALVAC recombinant containing HIV gp120TM (MN), gag/pro (IIIB) (C3 locus), Nef (C6 locus), and Pol (C5 locus), as described in U.S. application Serial No. 08/417,210, incorporated herein by reference.

15 Plasmids for preparing these recombinants were prepared as follows:

Vaccinia H4L Expression Cassette Into ALVAC

pCPM6LDEL was generated by using primer pair H4A and H4B to amplify a 900 bp fragment from pBAMM11.6 (ALVAC 11.6kb *Bam*HI M fragment in pBSSK vector background), and primer pair H4C and H4D (SEQ ID NOS:) to amplify a 940 bp fragment from pBAMM11.6 (H4C 5' ACTACTAATTAGCTATAAAAACCCGGGATTAGTTTATTACTAACTAATTACTATACTG3') (H4D 5' ATCATCGGATCCTTAATAATCTTATGAACCTTTATAAATATGAG3'). A fusion PCR reaction using the PCR products from the amplifications and primer pair H4A and H4D obtained an 1840 bp PCR fusion fragment which was then cloned into the T/A Cloning vector for sequence confirmation. The sequence was found to have a PCR deletion at position 30 8054. The 1840 bp fragment was removed from the T/A vector by digestion with *Bam*HI. The fragment was then cloned into the *Bam*HI digested pBSSK Δ *Eco*RI-*Sma*I vector. The deletion was repaired by digesting the construct with *Hind*III to remove a 250 bp fragment of the right arm and 35 religating to obtain pCPM6LDEL.

placZH6H4Lreverse was digested with *Psp*AI and *Asp*700 resulting in a 1920bp fragment containing the H6 promoter and the 5' 1780bp of the H4L gene. The remaining 590bp of the H4L gene were generated using PCR amplification from the plasmid template placZH6H4Lreverse using primer pair H4A and H4B. The oligonucleotide sequences for primer pair H4A and H4B (SEQ ID NOS:) are:

Oligonucleotide Sequences
10 H4A 5'- ATCATCGAACAGAGCTTCCGCTATCTGCATTAAAGTTT-3'
H4B 5'- ATCATCCCCGGGAAGCTTTAGTTATTGAAATTAATCATATA-3'

The 590bp PCR fragment was gel purified and cloned into the TA Cloning vector (Invitrogen San Diego, CA. 92121) for sequence confirmation. The 590bp insert 15 containing the 3' H4L sequences was excised from the TA vector by digestion with *Psp*AI and *Asp*700. The 1920bp and the 590bp fragments were directionally cloned into the *Psp*AI digested pCPM6LDEL plasmid vector (containing the deorfed ALVAC M6L insertion site) to generate the plasmid 20 pM6LDELH6H4 containing the H6/H4L expression cassette flanked by ALVAC sequences at the M6L insertion site.

ALVAC pC8 insertion vector was generated as follows: PCR J36, containing the C8 ORF and flanking sequences, was generated using JP121 (CAT-CAT-GAG-CTC- 25 ACT-TAT-TAC-ATC-CTA-CT) and JP122 (TAC-TAC-GGT-ACC-TTT-AAT-AAG-CAA-TCA-CT)

(SEQ ID NOS:) on ALVAC DNA. The resulting approximately 1.7 kb band was digested with *Asp*718/*Sac*I and ligated into *Asp*718/*Sac*I digested pBSSK+. After 30 confirmation by sequence analysis, the resulting plasmid was designated pCPF85S3L. To remove most of the C8 ORF and introduce transcriptional and translational stops along with a MCS into pCPF85S3L, the plasmid was digested with *Sna*BI/*Hind*III and ligated to ~115bp PCR J618I 35 *Sna*BI/*Hind*III fragment, yielding pC8. PCR J618I is a fusion PCR product of PCRs J616 and J617 using primers JP516 (TAG-GAA-GAT-ACG-TAT-TAT-TTT-ATA-C) and JP519 (ATC-

CCA-TTA-TGA-AAG-CTT-ATA-G). PCR J616 was generated using primers JP516 and JP517 (CTC-GAG-CTG-CAG-GAT-ATC-ATC-GAT-GGA-TCC-TTT-TTA-TAG-CTA-ATT-AGT-CAC-GTA-CCT-TTA-TCA-TTA-GTA-ACA-AAT) on plasmid pCPF85S3L. PCR J617 was
5 generated using primers JP518 (GGA-TCC-ATC-GAT-GAT-ATC-CTG-CAG-CTC-GAG-TTT-TTA-TGA-CTA-GTT-AAT-CAC-GGC-CGC-TCA-
ATA-TTG-TAT-TGG-ATG-GTT-AG) and JP519 on plasmid pCPF85S3L. Plasmid pC8, the C8 insertion plasmid, was confirmed by sequence analysis and contains a ~440bp left
10 arm, a ~1162bp right arm, a MCS with unique *Bam*HI, *Cla*I, *Eco*RV, *Pst*I, and *Xho*I sites, flanked by both transcriptional and translational stop sequences.

From the plasmid pM6LDELH6H4, the 2.5 Kbp H6/H4 expression cassette was excised with *Sma*I, and the
15 resulting 2.5 Kbp *Sma*I fragment was purified and inserted into the ALVAC pC8 insertion vector at the *Eco*RV site generating pC8H6H4.

K3L Expression Cassette

The K3L coding sequences were synthesized by
20 PCR amplification using pSD407VC containing Copenhagen vaccinia HindIII K fragment as template, as described in U.S. Patent No. 5,378,457. The oligonucleotides MPSYN 763 and MPSYN 764 (SEQ ID NOS:) were used as primers for the PCR reaction.

25 MPSYN 763

5' -

CCCTCTAGATCGCGATATCCGTTAAGTTGTATCGTAATGCTTGCATTTGTTATTC
GT-3'

MPSYN 764 5' - CCCGAATTCTATAAAATTATTGATGTCTACA-3'

30 The approximately 325bp PCR fragment was digested with *Xba*I and *Eco*RI yielding a 315bp fragment. This 315bp fragment was purified by isolation from an agarose gel and ligated with *Xba*I and *Eco*RI digested pBSSK+ vector (from Stratagene LA Jolla, CA.). The
35 nucleic acid sequence was confirmed directly from alkali denatured plasmid template as described in Hattori, M. and Sakaki, Y., 1986, using the modified T7 polymerase

(Tabor, S. and Richardson, C.C. 1987) and Sequenase (from U.S. Biochemicals Cleveland, OH.). This plasmid was designated pBS 763/764. Digesting pBS 763/764 with *NruI* and *XhoI*, a 340bp fragment was isolated for cloning into 5 the plasmid vector pMM154 containing a cassette with the vaccinia H6 promoter controlling an irrelevant gene in the NYVAC *tk* insertion vector background, which was prepared by digestion with *NruI* (partially) and *XhoI*, such that the 340bp fragment from pBS 763/764 containing 10 the K3L gene could be directionally oriented next to the H6 promoter generating pMPTKH6K3L. The plasmid pMP42GPT containing the dominant selectable marker Eco gpt gene (Pratt D. and Subramani S. 1983) under the control of the Entomopox 42k promoter, was digested with *SmaI* and *BamHI* 15 to yield a 0.7 Kbp 42k-Eco gpt expression cassette. This 0.7 Kbp fragment was purified and ligated into *SmaI* and *BamHI* cut pMPTKH6K3L generating the plasmid pMPTKH6K3Lgpt. This plasmid was digested with *XhoI*, generating a 1.2 Kbp fragment containing the H6/K3L and 20 the 42k/Ecogpt expression cassette, which was then gel purified. The 1.2 Kbp *XhoI* fragment was inserted into the *XhoI* site of the ALVAC C6 insertion plasmid pC6L (described in U.S. Patent No. 5,494,807), generating pMPC6H6K3Lgpt.

25 E3L/K3L ALVAC Expression Cassette

The entire E3L gene is contained within a 2.3 Kbp *EcoRI* fragment isolated from pSD401VC, which contained a clone of the *HindIII* E fragment from Copenhagen vaccinia. The 2.3 Kbp *EcoRI* fragment was 30 inserted into pMPC6H6K3Lgpt that had been partially digested with *EcoRI*, generating the plasmid pMPC6H6K3E3gpt. The plasmid pMPC6H6K3E3gpt was digested with *XhoI* and the resulting 6.8 Kbp vector fragment was purified and self-ligated, resulting in the plasmid 35 pMPC6E3. The plasmid pMPTKH6K3L was digested with *PspAI* and the resulting 560bp fragment containing the H6/K3L expression cassette was ligated into *PspAI* digested

pMPC6E3 resulting in the plasmid construct pMPC6H6K3E3.

Construction of the H6-promoted FHV gB donor plasmid

The entire coding region of the Feline Herpesvirus 1 glycoprotein gB (FHV-1 gB) was obtained by digestion of pJCA079 (FHV gB coding region in which 5' and 3' T_sNT sequences were mutated to change the early transcriptional stop signal without affecting amino acid sequences; the I3L vaccinia promoter has been coupled to the 5' end of the gB ORF; see Fig. 4, SEQ ID NO:) with PstI and isolating a 3 Kbp fragment from an agarose gel. The purified PstI fragment was cloned into an ALVAC C3 insertion plasmid (pVQH6CP3LSA) also digested with PstI (the unique BamHI site in pVQH6CP3LSA was previously inactivated by digestion with BamHI, blunting the ends with Klenow polymerase and religation; pVQH6CP3LSA was obtained by digesting pVQH6CP3L, discussed in U.S. Patent No. 5,494,807, with NotI and NsiI, from which a 6623 bp fragment was isolated and ligated to annealed oligonucleotides CP34 (5'GGCCGCGTCGACATGCA3') and CP35 (5'TGTCGACGC3') (SEQ ID NOS:). The resulting plasmid, pRAC5, was screened for proper orientation of the gB coding region with respect to the H6 promoter. To properly link the H6 promoter to the FHV gB initiation codon, an 800 bp PCR fragment was amplified from pJCA079 using oligonucleotides RG789 (SEQ ID NO:) (5'-TTTCATTATCGCGATATC-CGTTAACGTTGTATCGTAATGTCCACTCGTGGCGATC-3') and RG787 (SEQ ID NO:) (5'-GGAGGGTTTCAGAGGCAG-3'). This purified fragment was digested with NruI/BamHI and ligated into pRAC5 also digested with NruI/BamHI. The resulting plasmid was the FHV gB donor plasmid, pc3H6FHVB.

"String of Beads" Cassette

The "string of beads" expression cassette for the nef and pol CTL epitopes (H6/Pol 3/Nef C term/Pol 2/Nef central/Pol 1) was generated by PCR (polymerase chain reaction) as detailed below, using template pHXB2 for pol epitopes and template 2-60-HIV.3 for Nef

epitopes. Initial assembly was in two parts: (1) H6 (partial promoter)/Pol 3/Nef C term(Nef 2); (2) Pol 2/Nef central (Nef 1)/Pol 1 in pBSSK. These were combined, then moved to pBSH6-11 for the assembly of the 5 entire H6 promoter, then the H6/HIV cassette was moved to a C5 insertion plasmid.

(1) H6/Pol 3/Nef C term(Nef 2)

A 230 bp fragment (A) was derived by PCR to obtain the H6 linkage and Pol3 using synthetic 10 oligonucleotides MPSYN783 and MPSYN784 and template pHXBD2. pHXBD2 was derived at the NIH/NCI (Dr. Nancy Miller) from a recombinant phage library of *Xba*I digested DNA from HTLV-III infected H9 cells cloned in lambda-J1 (Shaw et al., 1994). This plasmid contains the entire 15 proviral DNA sequence of the HIV IIIB isolate.

A 110 bp fragment (B) was derived by PCR to obtain Nef2 using oligonucleotides MPSYN785/MPSYN786 and template p2-60-HIV.3 (described in U.S. application Serial No. 417,210).

20 PCR fragments A and B were combined in a PCR as template to obtain a 300bp fragment containing H6 linkage/Pol3/Nef2 using external primers MPSYN783/MPSYN786 (SEQ ID NOS:). The 300bp fragment was digested with *Xho*I/*Hind*III and a 290 bp fragment was 25 isolated and ligated with similarly digested pBSSK to generate pBS783/786. The sequence was confirmed.

(2) Pol 2/Nef central (Nef 1)/Pol 1

A 210 bp fragment (C) containing Pol2 was derived by PCR using synthetic oligonucleotides 30 MPSYN787/MPSYN788 (SEQ ID NOS:) and template pHXBD2.

A 270 bp fragment (D) containing Nef1 was derived by PCR using synthetic oligonucleotides MPSYN789/MPSYN790 (SEQ ID NOS:) and template p2-60-35 HIV.3 (described in U.S. application Serial No. 08/417,210).

A 170 bp fragment (E) containing Pol1 was derived by PCR using primers MPSYN791/MPSYN792 (SEQ ID

NOS:) and template pHXB2.

Fragments C and D were combined as template in a PCR for Pol 2/Nef 1 using external primers

MPSYN787/MPSYN790 (SEQ ID NOS:) resulting in a 460 bp

5 PCR product (C+D).

Fragments D and E were combined as template in a PCR for Nef 1/Pol 1 using external primers

MPSYN789/MPSYN792 (SEQ ID NOS:), resulting in isolation of a 420 bp fragment (D+E).

10 Fragments (C+D) and (D+E) were combined as template in a PCR with external primers MPSYN787/MPSYN792 (SEQ ID NOS:) to obtain a 610 bp fragment containing Pol 2/Nef 1/Pol 1. This 610 bp fragment was digested with *Hind*III/*Pst*I. The resulting 590 bp fragment was
15 ligated with pBSSK cut with *Hind*III/*Pst*I to generate pBS787/792. The sequence was confirmed.

MPSYN783: 5' CCC CTC GAG TCG CGA TAT CCG TTA AGT TTG TAT CGT AAT GCC ACT AAC AGA AGA AGC A 3' (58mer)

MPSYN784: 5' AAA TCT CCA CTC CAT CCT TGT TTT CAG ATT TTT 20 AAA 3' (36 mer)

MPSYN785: 5' AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT 3' (36 mer)

MPSYN786: 5' CCC AAG CTT ACA ATT TTT AAA ATA TTC AGG 3' (30 mer)

25 **MPSYN787:** 5' CCC AAG CTT ATG GCA ATA TTC CAA AGT AGC 3' (30 mer)

MPSYN788: 5' TGG AAA ACC TAC CAT GGT TGT AAG TCC CCA CCT CAA 3' (36 mer)

30 **MPSYN789:** 5' TGG GGA CTT ACA ACC ATG GTA GGT TTT CCA GTA ACA 3' (36 mer)

MPSYN790: 5' TAC AGT CTC AAT CAT TGG TAC TAG CTT GTA GCA CCA 3' (36 mer)

MPSYN791: 5' TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA 3' (36 mer)

35 **MPSYN792:** 5' CCC CCT GCA GAA AAA TTA AGG CCC AAT TTT TGA AAT 3' (36 mer)

(SEQ ID NOS:)

Assembly of entire cassette:

A 590 bp *Hind*III/*Pst*I fragment was isolated from pBS787/792 and ligated with vector pBS783/786 cut with *Hind*III/*Pst*I to generate pBS783/792. pBS783/792 was 5 cut with *Eco*RV and *Pst*I, to generate an 880 bp fragment which was then ligated with similarly digested vector pBSH6-1 to generate pBSH6PN. Plasmid pBSH6PN was digested with *Bam*HI and a 1060 bp fragment was isolated. pVQC5LSP1, a generic C5 donor plasmid, was digested with 10 *Bam*HI and ligated with the 1060 bp fragment from pBSH6PN. The resulting plasmid, pMPC5H6PN, contains the HIV pol/nef "string of beads" cassette in the ALVAC C5 locus.

Example 3 - Expression studiesExample 3.1 - NYVAC Expression Results

Dishes containing confluent monolayers of cells were infected at a multiplicity of infection (moi) of 2. After incubation for specified time periods, cells were incubated in labeling medium for 1 hour. At the end of 20 the incubation, cells were harvested for immunoprecipitation analysis as described (Harlow, E and Lane, D (1988) ; Langone, J. (1982)).

Cells were infected at an moi of 2 pfu/cell and incubated for specified time periods. At the appropriate 25 time post-infection, cell lysates were prepared for RNA analysis. The medium was aspirated and cells were harvested. RNA was isolated and prepared using the TRI-Reagent (Molecular Research Center Inc. Cincinnati, OH. 45212) as per manufacture instructions and analyzed by 30 slot blot. Radiolabelled DNA probes were used to detect specific RNA species.

The effect of vP1379 and vP1380 compared to the parental virus vP994 on the expression of HIV env truncated MN strain was studied by radiolabeling at 35 specific times post-infection on CEF cells. IP analysis with monoclonal antibody against HIV env truncated MN strain (mAb K3A) revealed a significant increase in de

novo synthesis for vP1380 infected cells at early times post infection compared to either vP994 parental virus or vP1379. A similar trend is observed at late times post infection. IP analysis with rabbit anti H4L antiserum (provided by Dr. S. Shuman, Sloan-Kettering Institute, NY) show that only vP1380 infected cells expressed H4L product early in infection. Neither vP994 nor vP1379 infected cells expressed H4L early in infection. All samples show de novo synthesis of H4L late in infection, but expression rates are higher for vP1380 infected cells than for either vP994 or vP1379 infected cells. IP analysis of E3L product, a constitutive vaccinia protein, show that de novo synthesis occurs at a higher rate at all times post infection in vP1380 infected cells than in either vP994 or vP1379 infected cells.

These results indicate that vP1379 is a defective recombinant with a pattern of expression identical to the parental virus unlike vP1380 recombinant which expresses H4L at early and late times post-infection. This early H4 expression clearly correlates with the enhanced expression of the proteins under study (HIV env and E3L) at early times post-infection.

The following studies were conducted with vP1380 and vP994 since vP1379 does not express H4L product at early times post-infection. The rate of expression at different times post infection in HeLa cells (non permissive system) was studied by IP analysis. IP analysis with anti-H4L shows that vP1380 infected cells expressed H4L product at all times post-infection (3, 6, 24 and 48 Hrs.). No product was detected in vP994 infected cells at any time post infection. Sustained de novo synthesis is observed that increases with time. Analysis of HIV Env product shows that, although product expression levels are higher at all times for vP1380 infected cells vs. vP994's, the most significant difference is seen at late times, 24 and 48 Hrs., suggesting that expression of H4L must have an impact at

some level on expression of HIV Env product. Expression of E3L product is also increased in vP1380 infected cells compared to vP994.

Experiments performed on L929 cells gave similar results. The most significant difference was that expression rates of the H4L product at all times post infection was very low, however there was a dramatic difference in the *de novo* synthesis rate of HIV Env component. Differences in the rates of Env synthesis peaked at 24 hours with a 5 to 10 fold increase in vP1380 infected cells compared to vP994.

Since H4L product is an early transcription factor, it is of interest to determine if the results obtained at the expression level correlate with an increase in H4L message in vP1380 infected cells. RNA analysis by slot blots indicate that H4L message is detectable at all times post infection in vP1380 infected cells and achieved a steady state at 6 Hrs. post infection.

HIV Env message levels increase rapidly to steady state levels at 3 Hrs post infection and remained at those levels for all time points in vP1380 infected cells. On the other hand, vP994 infected cells show a peak of HIV env message at 6 hours post infection and a decline starting at 12 hours. E3L message in vP1380 infected cells is present at higher levels for all times post infection compared to vP994 infected cells. This pattern of RNA levels is consistent with the pattern of *de novo* synthesis rate at the protein level.

Mice were immunized by the intraperitoneal route on day 0 and 28. Starting prior to the first immunization and at two week intervals following the immunization, mice were bled from the retroorbital plexus. Sera were prepared from the collected blood by standard clotting techniques and stored frozen at -20°C until use in kinetics ELISA for antibodies reactive to the HIV

envelope glycoprotein.

High doses of vP994 or vP1380 elicited similar levels of antibodies (Table below). However, at the lowest dose, 5×10^6 pfu, only vP1380 was capable of generating HIV antibodies. Moreover, the level of antibodies induced by the low dose was comparable to the levels of antibodies elicited by the highest dose, 5×10^7 pfu.

At a dose too low for vP994, lacking vaccinia H4 but identical in all other respects to vP1380, to elicit an antibody response, vP1380 induced antibody responses equivalent to those elicited by the highest doses tested. Thus, the overexpression of vaccinia H4L in NYVAC may result in increased potency for inducing humoral responses.

TABLE: Antibody responses to recombinant HIV-1 MN/BRU gp160.

5

KINETICS (mOD/min)

WEEKS

10

	VIRUS	DOSE	MOUSE	0	2	4	6	8	10	1214
15	NYVAC	HI	a	0	1	0	1	2	2	1 2
			b	0	0	0	2	2	2	2 2
			c	0	0	0	2	1	1	1 1
20	vP994 45	HI	a	0	6	8	42	45	44	44
	23		b	0	1	1	34	42	35	24
	34		c	3	1	3	34	40	31	33
25	vP994	LO	a	0	1	2	4	3	3	5 6
			b	1	1	0	2	2	3	3 3
			c	1	0	1	14	16	17	12
30	vP1380 50	HI	a	2	8	39	41	49	47	52
	49		b	3	12	45	49	46	51	54
	39		c	1	7	35	42	41	43	40
35	vP1380 44	LO	a	1	2	3	49	45	47	46
	40		b	0	1	2	30	30	34	36
	54		c	0	3	14	54	48	51	51

45

Mice were inoculated during weeks 0 and 4.
 vP994, HIV 1 MN gp140, noncleavable, secreted envelope glycoprotein.
 vP1380, HIV 1 MN gp140 + vaccinia H4L transcription factor.
 vCP125, HIV 1 MN gp160.
 HI dose, 5×10^7 pfu.
 LO dose, 5×10^6 pfu.

As discussed above, possibly, part of the enhanced levels in vP1380 are due to enhanced transcription and expression of viral specific products such as E3L, such that there is enhanced transcription and translation involved in expression in vP1380. There was more expression of the exogenous DNA and at more persistent levels in vP1380, in accordance with the invention wherein vectors obtain greater levels of expression and more persistent levels of expression. Enhanced expression profiles in the murine system provided enhanced immunogenicity in mice, as shown by vP1380 being more immunogenic in mice than vP994.

Another observation is that enhancement profiles are seen in restrictive early cells in the abortive early ALVAC recombinants herein, whereas the profiles were not observed in cells where there was productive replication,

5 e.g., VERO or CEF, suggesting that the factor and the foreign DNA preferably should be expressed substantially co-temporally or contemporaneously, i.e., that preferably there should be co-expression at substantially the same time or stage, and that the time of expression, e.g.,

10 early, late, early and late, should be matched with the phenotype of the vector (e.g., abortive early, abortive late), i.e., that in a system in which viral replication is not impaired (a permissive system) or in a system in which replication is aborted at a time when expression is

15 not matched with the phenotype of the vector may not obtain optimal expression. Thus, in an abortive early system such as ALVAC or NYVAC, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor early; in an abortive late

20 system, one preferably expresses exogenous DNA and a transcriptional or transcriptional and translational factor late or early and late (as expression only early may be akin to expression in a permissive system, i.e., one may not necessarily obtain optimal expression).

25 **Example 3.2 - ALVAC Expression Results**

ALVAC-HIV Recombinants

Immunoprecipitation (IP) was used to provide a semi-quantitative comparison of the temporal expression of the HIV-I cassette contained in the ALVAC recombinants

30 in MRC-5 infected cells. Heat inactivated sera from HIV patients was obtained and used for the IP as described in the methods. The antiserum will precipitate the 120 KDa env protein and the various cleavage products from the gag protein precursor. In the analysis of the IP data it

35 is apparent that the ALVAC recombinants vCP1431A and vCP1437A containing the E3L/K3L cassette had a significant increase in the level of expression at all

times post infection when compared to the ALVAC recombinant vCP205 without the E3L/K3L cassette.

Interestingly vCP1431A and vCP1437A had similar expression profiles; insertion of H6/H4L into an ALVAC 5 E3L/K3L background did not enhance expression above E3L/K3L, suggesting that vaccinia H4L is not necessarily functional in ALVAC; but, manipulation of ALVAC transcriptional factors would lead to enhanced expression. Although there are homologs of vaccinia 10 transcriptional factors in canarypox, the requirements in canarypox may be biochemically different; but, these differences can be ascertained by the skilled artisan without undue experimentation from this disclosure and the knowledge in the art. Furthermore, the present 15 invention provides *in vitro* systems for transcriptional analysis in canarypox or fowlpox using vaccinia virus.

RNA slot blots were used to evaluate temporal transcriptional expression in MRC-5 cells infected with the ALVAC recombinants vCP205 and vCP1431A and vCP1437A. 20 In this analysis comparisons were made to the levels of mRNA transcribed from the HIV-I cassette encoding the env and gag proteins. ALVAC recombinants containing the E3L/K3L cassette (vCP1431A and vCP1437A) did not exhibit a significant increase in the level of mRNA for the env 25 and gag genes above that of the ALVAC recombinant vCP205.

The previously discussed role E3L/K3L plays in the down regulation of PKR in vaccinia infected cells thereby modulating translation seems to be operative in the ALVAC recombinants containing the vaccinia E3L/K3L 30 functions. The data has shown that translation is significantly enhanced in cells infected with ALVAC recombinants containing the E3L/K3L genes, while no significant increase in the level of transcription has been detected. This exemplifies the impact of E3L/K3L 35 expression on translation efficiency in poxvirus infected cells.

Immunoprecipitation analyses were also

performed using radiolabeled lysates derived from CEF cells infected with ALVAC parental virus, ALVAC-MN120TMG (vCP205), ALVAC-MN120TMGNPst (vCP1433), vCP1452 and vCP300, as described previously (Taylor et al., 1990),
5 with human serum derived from HIV-seropositive individuals (anti-HIV). The analysis confirmed the expression of the envelope sequences with a molecular weight of 120kDa and the Gag precursor protein with a molecular weight of 55kDa in the recombinants but not in
10 the parental virus. However, vCP300 exhibits diminished expression in comparison to vCP1452, i.e., vCP1452 surprisingly demonstrates enhanced expression due to expression of transcription and/or translation factors, in accordance with the invention.

15 FAC scan analysis with the Human anti-HIV antibody demonstrated expression of gp120 on the surface of HeLa cells infected with ALVAC-MN120TMGNPst (vCP1433). No fluorescence was detected on cells infected with ALVAC parental virus.

20 Appropriate expression of the inserted HIV genes was further confirmed by immunoprecipitation analysis (using polyclonal serum pool from HIV infected individuals) performed on a radiolabelled lysate of MRC5 cells infected with vCP1433 or vCP1452. The analysis
25 confirmed the expression of the envelope sequences with a molecular weight of 120KDa and the Gag precursor protein with a molecular weight of 55 KDa in vCP1452.

vCP1452 had enhanced expression on human cells in comparison to vCP1433 and vCP300. Indeed, enhanced
30 expression was observed with the E3L/K3L translational factor in human and canine cells.

Preliminary immunogenicity studies in mice showed no evidence of enhanced immunogenicity by the E3L/K3L translational factor. This corresponds to no
35 observed enhanced expression in murine cells.

Furthermore, in murine cells, the limiting factor of ALVAC expression is at the transcription level.

Accordingly, use of an appropriate transcription factor can overcome the inability to observe enhanced expression in the murine system. Thus, the origin of the cell may be an important factor in *in vitro* or *in vivo* applications of the invention (note H4 data above), as may be the nature of the vector, e.g., the phenotype of the vector (e.g., abortive, and when abortive such as abortive early, abortive late); but, appropriate selection of a cell and vector phenotype and of time of expression of factor(s) and foreign and/or exogenous DNA are within the ambit of the skilled artisan, from this disclosure and the knowledge in the art, without undue experimentation.

ALVAC-FHV gB Recombinants

Analysis of the expression for vCP1459, vCP1460 and vCP1464 was accomplished by immunoprecipitation analysis using a sheep anti-FHV gB polyclonal sera. Human MRC-5 cells were inoculated at an moi =5 at time 0, and then pulsed for 1 hour with ^{35}S labelled methionine at times 3, 6, 24, 48 and 72 h p.i. The precipitated protein was separated on SDS-PAGE gels. Autoradiographs of these IPs were scanned using a densitometer. The methods used provide a semi-quantitative analysis of FHV gB expression at the specific time points.

Results show that all recombinants express the proper sized full-length, glycosylated FHV gB polypeptide (apparent MW of approximately 115 kDa). However, recombinants vCP1460 and vCP1464 show significant increase in the amount of gB protein (about 5 times) compared to vCP1459. In addition, these expression levels persist even at 72 hr p.i. Thus, it appears that the expression of vaccinia E3L/K3L in ALVAC has a significant effect on the level and persistence of FHV gB expression.

35 Example 4 - Additional Vectors

Using the documents cited herein and the teaching herein, including in the foregoing Examples,

plasmid and naked DNA vectors, and additional viral vectors, including poxvirus, e.g., NYVAC, TROVAC, ALVAC, MVA, ts (temperature sensitive) mutants, or early (DNA+) and late defective mutants, adenovirus, e.g., CAV such as

5 CAV2, herpesvirus, e.g., Epstein Barr, are generated with enhanced transcription or translation or transcription and translation, e.g., by using H4L, vaccinia D6, vaccinia A7, vaccinia G8R, vaccinia A1L, vaccinia A2L, vaccinia H5R (VLTF-1, -2, -3, -4, P3, VLTF-X) E3L, K3L,

10 VAI, EBER, sigma 3, TRBP, or combinations thereof to modify the vector to contain at least one transcription factor or at least one translation factor or at least one transcription factor and at least one translation factor; and accordingly, enhanced expression, of exogenous coding

15 nucleic acid molecules (such exogenous coding nucleic acid molecules including from documents cited herein or as otherwise known in the art, or from applying those teachings in conjunction with teachings herein) is obtained.

20 Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent

25 variations thereof are possible without departing from the spirit or scope of the present invention.

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WHAT IS CLAIMED IS:

1. A vector for enhanced expression of at least one first nucleic acid molecule in a cell having a particular phenotype, said vector modified to comprise
5 the first nucleic acid molecule and at least one second nucleic acid molecule encoding a transcription factor or a transcription factor and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to
10 the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by enhancing transcription or transcription and translation.

2. The vector of claim 1 wherein the first
15 nucleic acid molecule is operably linked to a first promoter and the second nucleic acid molecule is operably linked to a second promoter, and the first and second promoters are functional substantially co-temporally.

3. The vector of claim 2 wherein the first
20 and second nucleic acid molecules are at different loci within the vector.

4. The vector of claim 2 wherein the first and second nucleic acid molecules are at the same locus within the vector.

25 5. The vector of claim 1 wherein the first nucleic acid molecule and the second nucleic acid molecule are operably linked to a promoter.

6. The vector of claim 1 wherein transcription factor is of poxvirus origin.

30 7. The vector of claim 6 wherein the transcription factor is from a vaccinia virus.

8. The vector of claim 7 wherein the transcription factor is from an open reading frame selected from the group consisting of H4L, D6, A7, G8R,
35 A1L, A2L, H5R, and combinations thereof.

9. The vector of claim 1 wherein the second nucleic acid molecule is comprised of at least one

transcription factor and at least one translation factor.

10. The vector of claim 1 wherein the
translation factor effects inhibition of eIF-2 α
phosphorylation or inhibition of PKR phosphorylation or
5 otherwise sequesters dsRNA, increasing the effective
concentration of dsRNA.

11. The vector of claim 10 wherein said at
least one second molecule is selected from the group
consisting of: a K3L open reading frame, an E3L open
10 reading frame, a VAI RNA frame, an EBER RNA, a sigma 3
open reading frame, a TRBP open reading frame, and
combinations thereof.

12. The vector of claim 1 wherein said first
nucleic acid molecule encodes a molecule selected from
15 the group consisting of an epitope of interest, a
biological response modulator, a growth factor, a
recognition sequence, a therapeutic gene and a fusion
protein.

13. The vector of claim 1 which is a
20 recombinant virus.

14. The vector of claim 13 which is a
recombinant poxvirus.

15. A method for preparing a vector as claimed
in claim 1 comprising modifying the vector to comprise
25 the at least one second nucleic acid molecule, and
optionally also modifying the vector to comprise the
first nucleic acid molecule, so that there is
substantially co-temporal expression of the first and
second nucleic acid molecules with respect to the
30 phenotype of the cell.

16. The method for claim 15 comprising
operably linking the first nucleic acid molecule to a
first promoter and the second nucleic acid molecule to a
second promoter, wherein the first and second promoters
35 are functional substantially co-temporally.

17. The method for claim 15 comprising
operably linking the first and second nucleic acid

molecules to a promoter.

18. An immunological, vaccine or therapeutic composition comprising the vector of claim 1 and a pharmaceutically acceptable carrier or diluent.

5 19. A method for generating an immunological or therapeutic response in a host comprising administering to the host the composition of claim 18.

10 20. A method for increasing expression of at least one first nucleic acid molecule by a vector comprising the first nucleic acid molecule, wherein the expression is in a cell having a particular phenotype, and the method comprising modifying the vector to comprise at least one second nucleic acid molecule encoding a transcription factor or a transcription factor 15 and a translation factor, wherein there is substantially co-temporal expression of the first and second nucleic acid molecules with respect to the phenotype of the cell, whereby expression of the second nucleic acid molecule enhances expression of the first nucleic acid molecule by 20 enhancing transcription or transcription and translation.

21. A method for expressing a gene product *in vitro* comprising infecting, or transfecting, a suitable cell with a vector as claimed in claim 1.

22. A method for expressing the first nucleic acid molecule *in vivo* comprising administering the vector of claim 1 to a host.

23. vCP1452 or vCP1433.

1/22

FIG. 1

Fig. 1

Nucleotide sequence of the insert in vP1380 containing the mutagenized H4L orf and lacZ orf under the H6 promoter.

Characteristic	Position(s)
Left arm	1-798
Right arm	6636-7319
H6 promoter	C3307-3184 and C6495-6372
H4L orf	C3183-799
T to C mutations	C2836 and C2839
lacZ orf	C6371-3327

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1 GGATCCGCC GTTCTTATT TAGACCAAAA ATTGGTTTC ATGTTTCGA AGCGGTGTT
61 TGCACAAAGT CGGGGATCGT GTTCTACATA TTGGCGGCA TTATCCAGTA TCTGCCTATT
121 GATCTTCATT TCGTTTCGA TTCTGGCTAT TTCAAAATAA AATCCCGATG ATAGACCTCC
181 AGACTTATAA ATTTCATCTA CGATGTTCA CGCCGTAGTA ACITCTAATAA TATAGGTGTA
241 TAAGCTAAC A TCATACCTC CTGTATATGT GAATATGGTA TGATTTTGT CCATTACAAG
301 CTCGGTTTA ACTTTATTCG CTGTAATAAT TTCTCTCATC TGAGGATAT CTATTTTTT
361 GTCAATGCA TTCCATCAAGA CGGGACGAAG AAACGTAATA TCCCTAATAA CGTATCGTT
421 TTCTACAAATA ACTACATATT CTACCTTTT ATTTCTAAC TCGGTAAAAA AATTAGAATC
481 CCATAGGGCT AAAATGTCAG CGATATTCTC TTTCGTTCC TCTGTACACA TAGTGTACA
541 AAACCTGAA AAGAAGTGG TATACCTGTC ATCATTCTA ATGTTCCCTC CAGTCCACTG
601 TATAAACCCA TAATCCTGT AATGATCIGG ATCATCCCTG ACTACCCACAA CATTCTTTT
661 TTCTGGCATA ACITCGTGT CCTTACATC ATCGAACCTC TGATCAATTAA TATGCTCATG
721 AACATTAGGA AATGTTCTG ATGGAGGTCT ATCAATAACT GGCAACACAA TAACAGGAGT
781 TTTCACCGCC GCCAATTAGT TATTGAAATT AATCATATAC AACTCTTAA TAGGAGTAT
841 ATTTCTGCT ATCCATTGTT TCACATTAC ATATTCGAC AAAAGATAT AAAATGCGTA
901 TTCCAATGCT TCTCTGTTA ATGAAATTACT AAAATATACA AACACGTAC TGTCTGGCAA
961 TAAATGATAT CTTAGAATAT TGTAAACATT TATTTTGAT TGACACATGTT CGTGATCTAT
1021 GAGTCCTCT TCGAATGGCA TAGGATCTCC GAATCTGAAA ACITATAAT AGGAGTTAGA
1081 ATAATAATAT TTGAGAGTAT TGGTAATATA TAAACTCTT AGGGTATAA TTAGTTTTT
1141 TCTCTCAATT TCTATTTTA GATGTGATGG AAAATGACT AATTGTTAG CATTAGTATC
1201 ATGAACTCTA ATCAAAATCT TAATATCTTC GTCACACGT AGCTCTTGA AGTTTTAAG
1261 AGATGCATCA GTTGGTCTA CAGATGGAGT AGGTGCAACA ATTCTTGT CTACACATGT
1321 ATGTAATGGA GCCATGTTT TAACATATAAT GGTGCTTGT TCGAAAAACT TTAATGCGAGA
1381 TAGCGGAAGC TCTTCGCCG GACTTCTAC ATCGTAATIG GGTCTAACG CCGATCTCTG
1441 AATGGATACT AGTTTCTAA GTTCTAATGT GATTCTCTGA AAATGTAAT CCAATTCTC
1501 CGGCATTATA GATGTGTATA CATCGTAAA TAAACTATA GTATCCAACG ATCCCCTCTC
1561 GCAAATTCTA GTCTTAACCA AAAATCGTA TATAACCACG GAGATGGCGT ATTAAAGAGT
1621 GGATTCTCT ACCGTTTGT TCTTGGATGT CATATAGGA ACTATAAAGT CCGCACTACT
1681 GTTAAGAATG ATTTACTAACG CAACATATATA GTTCAAATTAA AGCATTTGG AAACATAAAA
1741 TAATCTGTA GACGATACCT GACTTCGAA TAAGTTGCA GACAAACGAA GAAAGAACAG
1801 ACCTCTCTTA ATTTCAGAAG AAAACCTT TTCGTTCTC TGACGCTAG AGTTTATATC
1861 AATAAGAAAG TTAAGAATTA GTCGGTTAAT GTTGTATTTC ATTACCAAG TTGAGATT
1921 CATAATATTA TCAAAAGACA TGATAATATT AAAGATAAAG CGCTGACTAT GAACGAAATA
1981 GCTATAAGGT TCGCTCAAAA ATATAGCTT GTTAAACGGG GAAACGATAA CTGTTTTTT
2041 AATCACGTCA CGGGCATCTA AATTAATAT AGGTATATT ATTCCACACA CTCTACAATA
2101 TGCCACACCA TCCTCTATAAT AAATAATTC GTTAGCAAA TTATTAATTT TAGTGAATAA
2161 GTTAGCGTCA ACITCTCATG CTTCCTCAA TCTAATTGTA TGCTCACACG GTGCGAATTIC
2221 TACTCTAAC A TCCCTTTTCC ATGCTCTCAGG TTTCATCGATC TCTATAATAT CTAGTTTTT
2281 GCGTTTCACA AACACAGGGT CGTCTCTCGC GATGAGATCT GTATAGTAAC TATGTAATG
2341 ATAACATGAT AGAAAGATGT AGCTATATAG ATGACGATCC TTTAAGAGAG GTATAATAAC
2401 TTTACCCCAA TCAGATAGAC TGTGTTATG GTCTTCGAA AAAGAATTTC TATAAATTTC

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FIG. 1 (cont'd.)

2461	TCCAGTATT	TCCAATATA	CGTACTTAAC	ATCTAAAAAA	TCCTTAATGA	TAATAGGAAT
2521	GGATAATCCG	TCTATTATC	AAAGAAATAC	ATATCGCACA	TTATACTTTT	TTTGGAAAT
2581	GGGAATACCG	ATGTGCTAC	ATAAATATGC	AAAGTCTAAA	TATTTTITAG	AGAATCTTAG
2641	TTGGTCCAAA	TTCTTTCCA	AGTACGGTAA	TAGATTITTC	ATATTGAACG	GTATCTCTT
2701	AATCTCTGGT	TCTAGTTCCG	CATTAATGA	TGAAACTAAG	TCACTATTTT	TATAACTAAC
2761	GATTACATCA	CCTCTAACAT	CATCATTTAC	CAGAACTACTG	ATCTCTTTT	GTCGTAATAA
2821	CATGTCTAAT	GTGTTGAAGA	AAAGATCATA	CAAGTTATAC	GTCATTICAT	CTGIGGTATT
2881	CTTGTCTATG	AAGGATAAAC	TCGTACTAAT	CTCTTCCTTA	ACAGCCCTGTT	CAAATTATA
2941	TCCTATATAC	GAAAAAAATAG	CAACCACTGT	TTGATCATCC	GCGTCAATAT	TCTGTTCTAT
3001	CGTAGTGTAT	AACAATCGTA	TATCTCTTC	TGTTGATAGTC	GATACGGTTAT	AAAGGTGAT
3061	AAAGAAAATA	TTTTTATTT	GTGAAATAAA	GTCATCGTAG	GATTGGAC	TTATATTGCG
3121	GTCTAGTAGA	TATGCTTTTA	TTTTGGAAT	GATCTCAATT	AGAATAGTCT	CTTATAGACTC
3181	CATTACGATA	CAAACCTAAC	GGATATCGCG	ATAATGAAAT	AATTATGAT	TATITCTCGC
3241	TTTCAATT	ACACAACCCCT	CAAGAACCTT	TGTATTTATT	TTCACIITTT	AAGTATAGAA
3301	TAAAGAAGCT	TCCCGGGGG	TCCCTATTTT	TGACACCAGA	CCAACIGGTA	ATGGTAGCGA
3361	CCGGCGCTCA	GCTGGAATT	CGCCGTAACT	GACGGGCTCC	AGGAGTCGTC	GCCACCAATC
3421	CCCCATATGGA	AACCGCTCGAT	ATTCAAGCCAT	GTGCCCTCTT	CCCGCGTGCAG	CAGATGGCGA
3481	TGGCTGGTT	CCATCAGTGT	CTGTTGACTG	TAGCGGCTGA	TGTGAACTG	GAAGTGGCG
3541	CGCCACTGGT	GTGGGCCATA	ATTCAATTG	CGCGTCCCGC	AGCGCAGACC	GTITTOGCTC
3601	GGGAAGACGT	ACGGGGTATA	CATGTCGAC	AATGGCAGAT	CCCAGCGTC	AAAACAGGGG
3661	GCAGTAAGGC	GGTGGGGATA	GTTTTCTTGC	GGCCCTAAATC	CGAGCCAGTT	TACCCGCTCT
3721	GCTACCTGGG	CCAGCTGGCA	GTTCAGGCCA	ATCCCGCGCG	GATGCGGTGT	ATCGCTCGCC
3781	ACTTCAACAT	CAACGGTAAT	CGCCATTG	CCACTACCAT	CAATCCGGTA	GGTTTTCCGG
3841	CTGATAAATA	AGGTTTCCC	CTGATGCTG	CAACGGTGAG	CGGTCGTAAT	CAGCACCGCA
3901	TCAGCAAGTG	TATCTGCCGT	GCACATGCAAC	AAOGCTGTT	CGGCCCTGGTA	ATGGCCCCGC
3961	GCCCTCCAGC	GTTCGACCCA	GGCGTAGGG	TCAATGCGG	TCGCTTCACT	TACGCCAATG
4021	TCGTTATCCA	GCGGTGACG	GGTGAACIGA	TCGCGCAGCG	GCGTCAGCAG	TTGTTTTTA
4081	TCGCCAATCC	ACATCTGIGA	AAGAAAGCCT	GACTGGCGGT	AAATTGCCA	ACCGCTTATTA
4141	CCCAGCTCGA	TGCAAAAATC	CATTTGCTG	GTGGTCAGAT	GCGGGATGGC	GTGGGACGCG
4201	GGGGGGAGCG	TCACACTGAG	GTTTTCCGG	AGACGCCACT	GCTGCCAGGC	GCTGATGTGC
4261	CCGGCTTCTG	ACCATGCGGT	CGCGTTCTG	TGCACTACGC	GTACTGIGAG	CCAGAGTTGC
4321	CCGGCGCTCT	CCGGCTGGG	TAGITCAGGC	AGTTCAATCA	ACTGTTTAC	TTGTTGGAGCG
4381	ACATCCAGAG	GCACITCACC	GCTGCGCAGC	GGCTTACCAT	CCAGCGCCAC	CATCCAGTGC
4441	AGGAGCTCGT	TATCGTATG	ACGGAACAGG	TATTCGCTGG	TCACTTCGAT	GGTTTGCCCC
4501	GATAAACGGA	ACTGGAAAAAA	CTGCTGCTGG	TGTTTCTT	CCGTCAAGCGC	TGGAIGCGGC
4561	GTGGGTGCGG	CAAAGACCGAG	ACCGTICATA	CAGAACCTGG	GATCGITCGG	CGTATOGCCA
4621	AAATCACCGC	CGTAAGCCGA	CCACGGGTG	CGGTTTTCAT	CATATTAAAT	CAGCGACTGA
4681	TCCACCCAGT	CCCAGGCAA	GCCGCCCTGT	AAACGGGGAT	ACTGACGAAA	CGCCCTGCCAG
4741	TATTTAGCGA	AAACGCCAAG	ACTGTTACCC	ATCGCTGGG	CGTATTGCGA	AAGGATCAGC
4801	GGGCGCGCT	CTCCAGGTAG	CGAAAGCCAT	TTTTGATGG	ACCATTTCGG	CACACGGGG
4861	AAAGGCTGGT	CTTCATCCAC	GCGCGCGTAC	ATCAGGGCAAA	TAATATCGGT	GGCCGIGGTG
4921	TCGGCTCCGC	CGCCTTCATA	CTGCACCGGG	CGGGAAGGAT	CGACAGAITT	GATCCAGCGA
4981	TACAGCGCGT	CGTGATTAGC	GCCGTGGCCT	GATTCAITCC	CCAGCGACCA	GATGATCACA
5041	CTCGGGTGT	TACGATCGCG	CTGCCACATT	CGCGTTACGC	GTCGCTCAT	CGCCGGTAGC
5101	CACCGGGGAT	CATCGGTCA	ACGATTCTT	GGCACCATGC	CGTGGGTTTC	AATATTGGCT
5161	TCATCCACCA	CATACAGGCC	GTAGCGTCG	CACAGCGTGT	ACCACAGCGG	ATGGTTCGGA
5221	TAATGCGAAC	AGGCACGGC	GTTAAAGTTG	TTCTGCTTC	TCAGCAGGAT	ATCCCTGCACC
5281	ATCGTCTGCT	CATCCATGAC	CTGACCATGC	AGAGGATGAT	GCTCGTIGACG	TTAACGCGCT
5341	CGAACATCAGCA	ACGGCTTGGCC	GTTCAGCAGC	AGCAGACCAT	TTCAATCCG	CACCTCGCGG
5401	AAACCGACAT	CGCAGGCITC	TGCTTCATC	AGOGTGGCGT	CGGCGGTGIG	CAGITCAACC
5461	ACCGCACGAT	AGAGATTGCG	GATTTGGCG	CTCCACAGT	TCGGGTTTTTC	GACGTTCAAGA
5521	CGTAGTGTGA	CGCGATCGGC	ATAACCCACCA	CGCTCATCGA	TAATTCACC	GCCGAAAGGC
5581	GGGGTGCGGC	TGGGACCTG	CGTTTCACTCC	TGCCATAAAAG	AAACTGTTAC	CCGTAGGTAG
5641	TCACGCAACT	CGCCGCACAT	CTGAACITCA	GCCTCCAGTA	CAGCGCGGCT	GAATATCATCA
5701	TTAAAGCGAG	TGGCAACATG	GAATACGCTG	ATTITGIGTAG	TCGGTTTATG	CAGCAACGAG
5761	ACGTCACTGA	AAATGCCGCT	CATCCGCCAC	ATATCCTGAT	CTTCCAGATA	ACTGCGCTCA
5821	CTCCAACGCA	GCACCATCAC	CGCGAGGCCG	TTTCTCCGG	CGCGTAAAAA	TGCGCTCAGG
5881	TCAAATTCTAG	ACGGCAAACG	ACTGTCCTGG	CGCTAACCGA	CCCAGCGCCC	GTTCGACCAAC
5941	AGATGAAACG	CCGAGITAAAC	GCCATCAAAA	ATAATTGCGC	TCTGGCTTC	CIGTAGCCAG
6001	CTTTCATCAA	CATTTAAATG	GAGCGAGTAA	CAACCCGTCG	GATTCTCGT	GGGAACAAAC
6061	GGCGGATGTA	CCGTAATGGG	ATAGGTTACG	TTGGTGTAGA	TGGGCCATC	GTAACCGTGC
6121	ATCTGCCAGT	TTGAGGGGAC	GACGACAGTA	TCGGCCTCAG	GAAGATCGCA	CTCCAGGCCAG

Fig. 1 (cont'd)

6181 CTTTCGGCA CGGCTTCTGG TGCCGGAAAC CAGGCAAAGC GCCATTGCC ATTCAAGGTG
6241 CGCAACTGTT GGGAAGGGCG ATCGGTGCGG GCCTCTCGC TATTAACGCC GCTGGCGAAA
6301 GGGGGATGTG CTGCAAGGCG ATTAAAGTTGG GTAAACGCCAG GGTTTTCCCA GTCACGACGT
6361 TGTAACCA TTACGATAAC AACTTAACCG ATATCGCGAT AATGAAATAA TTATGATTA
6421 TTTCGCGCTT TCAATTAAAC ACAACCCCTCA AGAACCTTGTG TATTTATTT CACTTTTAA
6481 GTATAGAATA AAGAACCCCG GAAGCTTGTC TAGCTGGTGC TGAGTTCTA CGTGAGTTGA
6541 TTCGTCCTT GCGTGCGCTT CGTGAATCCAA TTGTCGGAG ATATTCCTT CTCTTCGGT
6601 GCTTCITATG AAACTTCCC TGACGGTGGC GTTTTAAAGT TACAAACAAAC TAGGAAATIG
6661 GTTTATGATG TATAATTTT TTAGTTTTA TAGATTCTT ATTCTATAC TAAAAAAATGA
6721 AAATAAATAC AAAGGTTCTT GAGGGTTGTG TAAATTGAA AGCGAGAAAT AATCATAAAT
6781 TATTCATTA TCGCGATATC CGTTAAGTT GTATCGTAAT GGCGTGGTCA ATTACAATA
6841 AAGCGGATAC TAGTAGCTC ACAAAAGATGG CTGAAATCAG AGCTCATCTA AAAAATAGCG
6901 CTGAAAATAA AGATAAAAAC GAGGATAATT TCCCGGAAGA TGTAATAATT CCATCTACTA
6961 AGCCAAAAC CAAACGAGCC ACTACTCCCTC GTAAACCGC GGCTACTAAA AGATCAACCA
7021 AAAAGGAGGA AGTCCAAGAA GAAGTAGTTA TAGAGGAATA TCATCAAACA ACTGAAAAAA
7081 ATTCTCCATC TCCCTGGAGTC GGCGACATTG TAGAAAGCGT GGCTGCTGTA GAGCTCGATG
7141 ATAGGGACGG GGATGATGAA CCTATGGTAC AAGTTGAAGC TGGTAAAGTA AATCATAGTG
7201 CTAGAACCGA TCTTCCTGAC CTAAGGTGG CTACCGACAA TATCGTTAA GATCTTAAGA
7261 AAATTATTAC TAGAATCTCT GCAGTATCGA CGGTTCTAGA GGATGTTCAA GCAGGATCC

Fig. 2

Fig. 2

Nucleotide Sequence of the ALVAC C8 Insertion site containing the H6/H4L expression cassette

Characteristic	Position(s)bp
Left Arm	1-487
Right Arm	3016-4225
H6 Promoter	495-618
H4L cass	619-3003
1 GAGCTCACTT	ATTACATCCT ACTGACTATA TACAGCGAAT TAACCATAGG CGTAATTGTA
61 CAGAAACCCAG	GAAATTATTA CCGCCTTTA TAAGAAGTAT TAATAAAACA TGTAGCGTAT
121 GTCTAGAAAAG	AATATACGAA AAAGAAATAA ATAAACAATA TTTCGGTATT TTACCAAATT
181 GTAAACACCGT	GTTCGGTTC TACTGTATAC AACGGTGGAT GTCTATAATA AAAGGTACGG
241 ATACCGAAGG	TACATGCTCT GTATGTAGAA CAGTTCTGT ATTATAGTG CCTAATAGGT
301 ACTGGATAGA	CGATAAAAT GAAAAGAGAT TAATTATAAA TAAATATAAG AATGACAGAA
361 AGACTTAA	AGCGTTAA CATTATAGA GAAGATACGT ATTATTTAT ACAGTAAACA
421 ACAGTTTATT	TGTTACTAAT GATTAAGGTA CGTGA
481 CGATGATGGG	AAGCTTCTT ATTCTACTA TAAAAAGTGA AAATAAATAC AAAGGTTCTT
541 GAGGGTTGTG	TTAAATTGAA AGCGAGAAAT AATCATAAT TATTCATTA TCGCGATATC
601 CGTTAAGT	GTATCGTAA GGACTCTAA GAGACTATTC TAATTGAGAT CATTCCAAA
661 ATAAAAGCAT	ATCTACTAGA CGCGAATATA AGTCCAAAT CCTACGATGA CTTTATTTC
721 CGAAATAAAA	ATATTTTCGT TATCAACCTT TATAACGTAT CGACTATCAC AGAAGAACAT
781 ATACGATTGT	TATACACTAC GATAGAACAG AATATTGACG CGGATGATCA AACACTGTT
841 GCTATTTTT	CGTATATTAG ATATAAATTG GAACAGGCTG TTAAAGAAGA GATTAGTACG
901 AGTTTACCT	TCAATGACAA GAATACCAAA CATGAAATGA CGTAACTT GTATGATCTT
961 TTCTTCAACA	CATTAGACAT GTATTACGA CAAAAGAAGA TCAGTATTCT GGTAAATGAT
1021 GATGTTAGAG	GTGATGTAAT CGTTAGTTAT AAAAATAGTGC ACTTAGTTTC ATCATTTAAT
1081 GCGGAACCTAG	AACCAGAGAT TAAGAAGATA CCGTTCAATA TGAAAAATCT ATTACCGTAC
1141 TTGAAAAGA	ATTTGGACCA ACTAAGATTG TCTAAAAAAT ATTTAGACTT TGCATATTTA
1201 TGTAGACACA	TCGGTATTC CATTCCAAA AAAAGTATA ATGTGCGATA TGTTATTCTT
1261 TATAAAATAG	ACGGATTATC CATTCTTATC ATCATTAAAGG ATTTTTAGA TGTTAAGTAC
1321 GTATATTG	AAAATACTGG AAAAATTAT TTTCCGAAGA CCATAACAAAC
1381 AGCTTATCTG	ATTGGGTAA AGTTATTATA CCTCTCTTAA AGGATCGTCA TCTATATAGC
1441 TACATCTTC	TATCTAGTT TCATTACAT AGTTACTATA CAGATCTCAT CGCGAGAGAC
1501 GAGCTGTGT	TTGTGAAACG CAAAAAAACTA GATATTATAG AGATCGATGA ACCTGAGGCA
1561 TGGAAAAGGG	ATGTTAGAGT AGAATTGCGA CCGTGTGAGC ATCAAATTAG ATGAAAGGAA
1621 GCTATGAAAG	TTGACGCTAA CTATTCACT AAAATTAAATA ATTTGCTAA CGAATTATT
1681 TATTATGAG	ATGGTGTGGC ATATTGAGA GTGTGTGGAA TAAATATACC TATATTTAAT
1741 TTAGATGCCG	CTGACGCTGAT TAAAATACA CTTATCGTTT CCACGTTAA CAAGACTATA
1801 TTTTGAGCG	AACCATATAG CTATTCTGTT CATAGTCAGC GCTTATCTT TATATTATC
1861 ATGTTTTTG	ATAATATTAG GAAATCTAA ACTTGGGTTA TGAAATACAA CATTAACCGA
1921 CTAATTCTTA	ACTTTCTTAT TGATATAAAC TCTAGACGTC AGGAATACGA AAAAAGTTT
1981 TCTCTGAAA	TTAAGAGAGG TCTGTTCTT CTCGTTCTG CTGCAAACCTT ATTCGAAAGT
2041 CAAGTATCGT	CTACAGAGTT ATTTATGTT TCCAAAATGC TTAATTGAA CTATATAGTT
2101 GCGTTAGTAA	TCATTCTAA CAGTAGTCGC GACTTTATAG TTTCTATAT GACATCCAAG
2161 AACAAAACGG	TAGAAGAATC CACTCTAAA TACGCCATCT CCGTGTGTTAT ATACGATTT
2221 TTGGTTAAGA	CTAGAATTG CGAGAAGGGA TCGTTGGATA CTATAGTTT ATTAACCGAT
2281 GTATACACAT	CTATAATGCC GGAGGAATTG GATTTACATT TTCAGAGAAT CACATTAGAA
2341 CTTAGAAAAAC	TAGTATCCAT TCAGAGATCG CGCTTGAAC CCAATTACGA TGTAGAAAGT
2401 CGCGCGCAAG	AGCTTCCGCT ATCTGCATTA AAGTTTTTCG ATACAAGCAC CATTATAGTT
2461 AAAACAATGG	CTCCAGTACA TACATGTGA GAACAAAAAA TTGTTGCACC TACTCCATCT
2521 GTAGAACCAA	CTGATGCATC TCTTAAACAC TTCAGAACGTC TAACGTGTGA CGAAGATATT
2581 AAGATTTGA	TTAGAGTCA TGATACTAAT GCTACAAAAT TAGTCATTT TCCATCACAT
2641 CTAAAAATAG	AAATTGAGAG AAAAAGACTA ATTATACCGC TAAAGAGTTT ATATATTAC
2701 AATACTCTCA	AATATTATTA TTCTAATCC TATTTATACG TTTTCAGATT CGGAGATCCT
2761 ATGCCATTG	AGAAGAACT CATAGATCAC GAACATGTGC AATACAAAAT AAATTGTTAC
2821 AATATTCTAA	GATATCATT ATTGCCAGAC AGTGCAGTGT TTGTATATT TAGTAATTCA
2881 TTAACAGAG	AGGCAATTGGA ATACGCATT TATATCTTT TGTCGAAATA TGAAATGTG
2941 AAACAATGGA	TAGACGAAAA TATAACTCGT ATTAAGAGT TGTATATGAT TAATTCAAT
3001 AACTAAAAGC	TTCCCATCCT GCAGCTCGAG TTTTATGAC TAGTTAATCA CGGCCGCTCA

Fig. 2 (cont'd)

3061 ATATTGTATT GGATGGTTAG AGATCAAAGG ATACAAGATA ACTGGGCTCA TTTCAGCTT
3121 ACATTCTACCC CTATAAGCTT TCATAATGGG ATTTTCTCC ATAATGTCAA AATCACTTTG
3181 GATATATTCA AAATTTCTA CAAAATGTT TGTTGTTCT GAGCTAAACA CGATGTTAGA
3241 TATTAATAAC TTTGCTATCT CAAGACCTTC TGAAGTATCA ACTTTGATAT TGGAAAGAGG
3301 TGAAAATAA GGTGATGAAG CGATTGTTCT ATCTGCACAG AATGTTAAC A GTATATCTAC
3361 TAATTCTACA TTTCCATCTG TCACAGCATC CCATAGAGGA GTATTCCAGT ACCTGTCCTT
3421 AGCATTATA TCAGCACCGA ATTCAAAAG CATAATAGTT ATCTTACAG ATCCTATACA
3481 CACAGCATAA TGCAAAGGAG TCATCCTATC GCTATCTTA ACGTTAGTAT ATGCTCCAGC
3541 TAGAAGTAAT TGCTCTATTA TCTCCATGTT TTCAGATTAA ACAGCATAAT GCAATGGATA
3601 CATATATCCT CTGTAACCAT AATTTATACT CGATCCAGCT TTTAGTAACA TACTCACAAT
3661 TTCCAATTTC TCTCTCTTA TAGCCTCGAT TATGGGATGA TTTCCCTGT ACTCATTGTC
3721 AACATCAGCG TTATACTCCA GAAGTAACTT TACAATTCC ACATTCTCTA TAGAGACAGC
3781 ATACTGGAGT GGAGTCTTTA CTTTGAGTC CTCATATGTA TCCACATTAG CGCCATGATC
3841 CAACAAGAGT TTCACCAGAT CTATGTTCTG AACTTTGACA GCTCTATGCA ACGGAGAAGA
3901 TACTTGTTCG CTAGATATAT CAGGATCAGC TCCGTGTAAC AATAGAGCTT TGGCTATTT
3961 AAATTTTCA TTTCTACAG CACAATGAAG GGGTGACAG CCATAATCGT TGAATACGTC
4021 CAGGTTAATG CCGGTTTCA CAATATCTAG CACGCTAGAC AGAGATCCAG ATTCAATAGC
4081 TTCGAATAAG TATGCCCTCA TTTGTGTAAG TAGTAGTAAG TAATAATTTC CTGAAGAAC
4141 TACTAACTTA CCGAGCTATA GTAGATAGTT ATAATTCTAT TTTTTACAA GTAGTATCAC
4201 ATAGTGATTG CTTATTAAG GTACC

Fig. 3

Fig. 3

Nucleotide sequence of the ALVAC C6 insertion site containing the H6 / K3L and E3L expression cassette.

Characteristic	Position(s)
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Left Arm	1-385
Right Arm	3273-4434 ↙
K3L orf	C727-464
H6 Promoter	C850-728
E3L	C2758-2188

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1 GAGCTCGCGG CCGCTATCA AAAGTCITAA TGAGTTAGGT GTAGATAGTA TAGATATTAC
61 TACAAAGGTA TTCATATITC CTATCAATTIC TAAAGTAGAT GATATTAATA ACTCAAAGAT
121 GATGATAGTA GATAATAGAT ACGCTCATAT AATGACIGCA AATTGGACG GTTACACATT
181 TAATCATCAC GCGTTCATAA GTTCAACTG CATAGATCAA AATCTCACTA AAAAGATAGC
241 CGAIGTATTG GAGAGAGATT GGACATCTAA CTACGCTAAA GAAATTACAG TTATAAATAA
301 TACATAATGG ATTTGTTAT CATCAGTAT ATTTAACATA AGTACAATAA AAAGTATTAA
361 ATAAAAATAC TTACTTACGA AAAATGACT AATTAGCTAT AAAAACCCAG ATCTCTCGAG
421 GTCGACGGTA TCGATAAGCT TGATATCGAA TTCATAAAA TTATTGATGT CTACACATCC
481 TTTTGTAAATT GACATCTATA TATCCTTTTG TATAATCAAC TCTAATCACT TTAACCTTTA
541 CAGTTTCCC TACCACTTAA TCCCTATATC CAACATATCT ATCCATATGC ATCTTAACAC
601 TCTCTGCCAA GATAGCTTC GAGTGAGGAT AGTCAAAAG ATAAATGTAT AGAGCATAAT
661 CCTCTCTCGTA TACTCTGCC TTTATTACAT CGCCCGCATT GGGCAACGAA TAACAAAATG
721 CAAGCATACG ATACAAACIT AACGGATATC GCGATAATGA AATAATTAT GATTATTTCT
781 CGCTTCAAT TTAACACAAC CCTCAAGAAC CTTTGTATT ATTTCACIT TTAAAGTATA
841 GAATAAGGAA AGCTCTAATT ATTAATGAA CAGATGTTT CGTTTCCCC TTGGCGTATC
901 ACTAATTAAAT TAACTGGGC TGCACTCGA GGAATTCAC TATATCGACA TATTTCATT
961 GTATACACAT AACCAATTCT AACGTTAGAAT GTATAGGAAG AGATGTAACG GGAACAGGGT
1021 TTTTGATTTC GCAAACATT TTAATACATA ATTCTCTGT TAATACGTCT TGCACTGAAT
1081 CTATTATAGA TGCCAAGATA TCTATATAAT TATTGTAA GATGATGTTA ACTATGTGAT
1141 CTATATAAGT AGTGTAAATAA TTCACTGTATT TCGATATATG TTCCAACCTCT GTCTTTGTGA
1201 TGCTCTGTTT CGTAATATCT ATAGCATCT CAAAAAATAT ATTGCATAT ATTCCCAAGT
1261 CTTCAGTCT ATCTCTCAA AAATCTTCAA CGTATGGAAT ATAATTAATCT ATTTCACCTC
1321 TTCTGATATC ATTAATGATA TAGTTTTGTA CACTATCTC TGTCAATTGA TTCTTATTCA
1381 CTATATCTAA GAAACGGATA GCGTCCCTAG GACGAACCTAC TGCCATTAT ATCTCTTATTA
1441 TAGCTTCTGG ACATAATTCA TCTATTATAC CAGAATTAAAT GGGAACTATT CCGTATCTAT
1501 CTAACATAGT TTTAAGAAAG TCAGAATCTA AGACCIGATG TTCAATATT GGTTCATACA
1561 TGAATATGTC TCTATGATG ATAGTGACTA TTTCATTCTC TGAAAATTGG TAACTCATT
1621 TATATATGCT TTCTCTGTT ATGAAGGATA GAATATACTC ATAGAATT GTACCAACAA
1681 ACTGTTCTCT TATGAATCGT ATATCATCT CTGAAATAAT CATGTAAGGC ATACATTAA
1741 CAAATTGAGA CTGCTCTCT GTTATCAATA TACTATTCTT GTGATAATT ATGIGTGGG
1801 CAAATTGTC CACGTCCTT AATTGTTGA TAGTAGATAT CAAATCCAT GGAGCTACAG
1861 TTCTGGCTT AAACAGATAT AGTTTTCTG GAACAAATT TACAACATTA TTATAAAGGA
1921 CTTGGGTAG ATAAGTGGGA TGAAATCTA TTTTAATTAA TGCTATCGA TTGTCCTCGT
1981 GCAAATATCC AAACGCTTT GTGATAGTAT GGCATTCTT GTCTAGAAC GCTCTACGA
2041 TATCTGIGAC AGATATCTC TTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATT
2101 GTATTTTAAAT ATCTATCTCA ATAAAAAAAT TAATAATGT GATTCAATGT ATAACTAAAC
2161 TACTAACIGT TATTGATAAC TAGAATCAGA ATCTAATGT GACGTAACCA AGAAGTTAT
2221 CTACTGCCAA TTAGCTGCA TTATTTTAG CATCTCGTT AGATTTCCTA TCTGCCATT
2281 CGAAACTCTC TCCGTCGATG TCTACACAGG CATAAAATGT AGGAGAGTTA CTAGGCCAA
2341 CTGATTCAAT ACGAAAAGAC CAATCTCTC TAGTTATTG GCAGTACTCA TTAATAATGG
2401 TGACAGGGTT AGCATCTTC CAATCAATAA TTTTTTAGC CGGAATAACA TCATCAAAG
2461 ACTTATGTC CTCTCTCATT GATTTTCGC GGGATACATC ATCTATTATG ACGTCAGCCA

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Fig. 3 (cont'd)

2521 TAGCATCAGC ATCCGGCTTA TCCGCCTCGG TTGTATAAA CCAACGAGGA GGAATATCGT
 2581 CGGAGCTGTA CACCATAGCA CTACGTTGAA GATCGTACAG AGCTTATTAA ACTTCCTCGCT
 2641 TCTCCATATT AAGTTGTCTA GTTAGTGTG CAGCAGTAGC TCCTTCGATT CCAATGTTTT
 2701 TAATAGCCGC ACACACAATC TCTGGTCAG AACGCTCGTC AATATAGATC TTAGACATT
 2761 TTAGAGAGAA CTAACACAAAC CAGCAATAAA ACTGAACCTA CTTTATCATT TTTTATICA
 2821 TCATCCTCTG GTGGTTCGTC GTTCTATCG AAATGTAGCTC TGATTAACCC GTCATCTATA
 2881 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT
 2941 ATTTCTCTGT TTTCATGTAT CGATTGCGTT GTAACATTAA GATTGCGAAA TGCTCTAAAT
 3001 TTGGGAGGCT TAAAGTGTIG TTGCAATCT CTACACGCGT GTCTAATAG TGGAGGTTCTG
 3061 TCAGCTGCTC TAGTTGAAT CATCATCGGC GTAGTATTCC TACTTTTACA GTTGGACAC
 3121 GGIGTATIGT ATTTCTCGTC GAGAACGTT AAATAATCGT TGTAACICAC ATCCTTTATT
 3181 TTATCTATAT TGTATTCTAC TCCCTTCCTA ATGCATTTTA TACCGAATAA GAGATAGCGA
 3241 AGGAATTCTT TTATTGATT AACTAGTCAA ATAGATATAT ATAATIGAAA AAGTAAAATA
 3301 TAAATCATAT AATAATGAAA CGAAATATCA GTAAATAGACA GGAACIGGC AATTCTCTT
 3361 CTAATGAAGT AAGTACTGCT AAATCTCCA AATTAGATAA AAATGATACA GCAAATACAG
 3421 CTTCATTCAA CGAATTACCT TTAAATTTT TCAGACACAC CTTAACTCAA ACTAACTAAG
 3481 TCAGATGATG AGAAAAGTAAA TATAAATTAA ACTTATGGGT ATAATATAAT AAAGATTCT
 3541 GATATTATAA ATTAACTTAA CGATGTTAAT AGACITATTIC CATCAACCCC TTCAAACCTT
 3601 TCTGGATATT ATAAAAATACC AGTTAATGAT ATTTAAATAG ATIGTTAAG AGATGAAAT
 3661 AATTATTGAG AGGTAAGGA TATAAAATTG GTCCTATCTT CACATGGAAA TGAATTACCT
 3721 AATAATTATAA ATTATGATAG GAATTTTTA GGATTTACAG CTGTTATAIG TATCAACAAT
 3781 ACAGGCAGAT CTATGGTTAT GGAAAACAC TGTAAACGGGA AGCAGCATTC TATGGTAACT
 3841 GGCCTATGTT TAATAGCCAG ATCATTTTAC TCCTATAAACCA TTTTACCA AATAATAGGA
 3901 TCCTCTAGAT ATTTAATATT ATATCTAAC ACAACAAAAA AATTAAACGA TGTATGGCCA
 3961 GAAGTATTT CTACTAATAA AGATAAAGAT AGTCTATCTT ATCTACAAGA TATGAAAGAA
 4021 GATAATCATT TAGTAGTACG TACTAATATG GAAAGAAAAG TATACAAAAA CGTGGAAAGCT
 4081 TTATATTAA ATAGCATATT ACTAGAAGAT TTAAAATCTA GACTTAGTAT AACAAAACAG
 4141 TTAAATGCCA ATATCGATTC TATATTTCAT CATAAACAGTA GTACATTAAT CAGTGATATA
 4201 CTGAAACGAT CTACAGACTC AACTATGCAA GGATAAACCA ATATGCCAAT TATGCTAAAT
 4261 ATTTTAACCT TAGAACTAAA ACGTTCTACC AATACTAAAA ATAGGATACG TGATAGGCTG
 4321 TTAAAAGCTG CAATAAAATAG TAAGGATGTA GAAGAAATAC TTGTTCTAT ACCCTGGAG
 4381 GAAAGAACTT TAGAACAACT TAAGTTAAT CAAACTTGTA TTTATGAAGG TACC

Fig. 4

Figure 4 DNA sequence of the coding region of FHV gB with modified T5NT motifs.

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1 ATGTCCACTC GTGGCGATCT TGGGAAGCGG CGACGAGGGA GTCGTTGGCA GGGACACAGT
61 GGCTATTTC GACAGAGATG TTTTTTCCCT TCTCTACTCG GTATTGCAGC GACTGGCTCC
121 AGACATGGTA ACGGATCGTC GGGATTAACC AGACTAGCTA GATATGTTTC ATTATCTGG
181 ATCGTACTAT TCTTAGTCGG TCCCCGTCCA GTAGAGGGTC AATCTGGAAG CACATCGGAA
241 CAACCCCGGC GGACTGTAGC TACCCCTGAG GTAGGGGTA CACCACAAA ACCAACTACA
301 GATCCCACCG ATATGTCGGA TATGAGGGAA GCTCTCCGT CGTCCCAAAT AGAGGCTAAC
361 GGACCACCGA CTTTCTATAT GTGTCCACCA CCTTCAGGAT CTACTGTCGT GCGTTTAGAG
421 CCACACCGGG CCTGTCAGA TTATAAACTA GGGAAAAATT TTACCGAGGG TATAGCTGTA
481 ATATTTAAAG AAAATATAGC CCCATATAAA TTCAAGGAA ATATATACTA TAAAAACATT
541 ATTATGACAA CGGTATGGTC TGGGAGTTCC TATGCCGTTA CAACCAACCG ATATACAGAC
601 AGGGTTCCCG TGAAAGTTCA AGAGATTACA GATCTCATAG ATAGACGGGG TATGTGCCTC
661 TCGAAAGCTG ATTACGTTCG TAACAATTAT CAATTACGG CCTTGATCG AGACGAGGAT
721 CCCAGAGAAC TGCCCTCTGAA ACCCTCCAAG TTCAACACTC CAGAGTCCCG TGGATGGCAC
781 ACCACCAATG AAACATACAC AAAGATCGGT GCTGCTGGAT TTCACCACTC TGGGACCTCT
841 GTAAATTGCA TCGTAGAGGA AGTGGATGCA AGATCTGTAT ATCCATATGA CTCATTGCT
901 ATCTCCACTG GTGACGTGAT TCACATGTC CCATTCTTG GGCTGAGGGG TGGAGCCCAT
961 GTAGAACATA CTAGTTATTTC TTCAGACAGA TTTCAACAAA TCGAGGGATA CTATCCAATA
1021 GACTTGGATA CGCGATTACA ACTGGGGGCA CCAGTTCTC GCAATTTC GAAAACTCCG
1081 CATGTGACAG TGGCCTGGAA CTGGACCCCA AAGTCTGGTC GGGTATGTAC CTTAGCCAAA
1141 TGGAGGGAAA TAGATGAAAT GCTACGCGAT GAATATCAGG CCTCCTATAG ATTACAGCC
1201 AAGACCATAT CCGCTACTTT CATCTCAAT ACTTCACAAT TTGAAATCAA TCGTATCCGT
1261 TTGGGGGACT GTGCCACCAA GGAGGCAGCC GAAGCCATAG ACCGGATTAA TAAGAGTAAA
1321 TATAGTAAAA CTCATATTCA GACTGGAACC CTGGAGACCT ACCTAGCCCG TGGGGATT
1381 CTAATAGCTT TCCGTCCCAT GATCAGCAAC GAACAGCAA AGTTATATAT CAATGAATT
1441 GCACGTCTCA ATCGCAGGT AGATCTCAGT GCACTCTCA ATCCATCTGG GAAACAGTA
1501 CAACGAACTA GAAGATCGGT CCCATCTAAAT CAACATCATA GGTGCGGGCG CAGCACAAATA
1561 GAGGGGGGTA TAGAAACCGT GAACAATGCA TCACTCTCA AGACCACCTC ATCTGTGAA
1621 TTCGCAATGC TACAATTTCG CTATGACTAC ATACAAGCCC ATGTAATGAA ATGTTGAGT
1681 CGGATAGCCA CTGCGCTGGTG TACACTTCA AACCAGGAAC ATGTCGCTG GACAGAGACC
1741 CTAAAACCTCA ATCCCCGGTGG GGTGGTCTCG ATGGCCCTAG AACGTCGTG ATCCGCGCGC
1801 CTACTTGGAG ATGCCGTCCG CGTAACACAA TGTGTTAACAA TTTCTAGCGG ACATGTCTAT
1861 ATCCAAAATT CTATGCGGGT GACGGGTTCA TCAACGACAT GTTACAGCCG CCCTCTTGT
1921 TCCCTCCGTG CCCTCAATGA CTCCGAATAC ATAGAAGGAC AACTAGGGGA AAACAATGAA
1981 CTTCTCGTGG AACGAAAATT AATTGAGCCT TGCACGTCA ATAATAAGCG GTATTTAAG
2041 TTTGGGGCAG ATTATGTATA TTTTGAGGAT TATGCGTATG TCCGTAAAGT CCCGCTATCG
2101 GAGATAGAAC TGATAAGTGC GTATGTGAAT TTAAATCTTA CTCTCCTAGA GGATCGTGA
2161 TTTCTCCAC TCGAAGTTTA TACACGAGCT GAGCTGGAAG ATACCGGCCT TTTGGACTAC
2221 AGCGAGATTG AACGCCGCAA CCAACTCCAC GCCTTAAAT TTTATGATAT AGACAGCATA
2281 GTCAGAGTGG ATAATAATCT TGTCATCATG CGTGGTATGG CAAATTCTT TCAGGGACTC
2341 GGGGATGTGG GGGCTGGTTT CGGCAAGGTG GTCTTAGGGG CTGCGAGTGC GGTAATCTCA
2401 ACAGTATCAG CGTATCATC ATTTCTAAAC AACCCATTG GAGCATTGGC CGTGGGACTG
2461 TTAATATTAG CTGGCATCGT CGCAGCATTG CTGGCATATC GCTATATATC TAGATTACGT
2521 GCAAATCCAA TGAAAGCCTT ATATCCTGTG ACGACTAGGA ATTTGAAACA GACGCTAAGA
2581 GCCCCCTCAA CGGCTGGTGG GGATAGCGAC CCGGGAGTCG ATGACTTCGA TGAGGAAAAG
2641 CTAATGCAGG CAAGGGAGAT GATAAAATAT ATGTCCTCG TATCGGCTAT GGAGCAACAA
2701 GAACATAAGG CGATGAAAAA GAATAAGGGC CCAGCGATCC TAACGAGTCA TCTCACTAAC
2761 ATGGCCCTCC GTCGCCGTGG ACCTAAATAC CAACGCCCTCA ATAATCTGA TAGCGGTGAT

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WO 98/40501

PCT/US98/02669

9/22

Fig. 4 (co +d)

2821 GATACTGAAA CAAATCTTGT CTAA

Fig. 5

Figure 5 DNA sequence of the H6 promoted FHV gB donor plasmid pC3H6FHVb.

H6 promoter: 3958 - 3835

FHV gB coding region: 3834 - 991

C3 left arm: 15 - 939

C3 right arm: 4056 - 6628

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1 GCGGCCGCGT CGACATGCAT TGTTAGTTCT GTAGATCAGT AACGTATAGC ATACGAGTAT
61 AATTATCGTA GGTAGTAGGT ATCCCTAAAAT AAATCTGATA CAGATAATAA CTTTGTAAAT
121 CAATTCAGCA ATTCTCTAT TATCATGATA ATGATTAATA CACAGCGTGT CGTTATTTT
181 TGTTACGATA GTATTTCTAA AGTAAAGAGC AGGAATCCCT AGTATAATAG AAATAATCCA
241 TATGAAAAAT ATAGTAATGT ACATATTCT AATGTTAAC TATTTATAGG TAAATCCAGG
301 AAGGGTAATT TTACATATC TATATACGCT TATTACAGTT ATTAAAAATA TACTTGCAA
361 CATGTTAGAA GTAAAAAAGA AAGAACTAAT TTTACAAAGT GCTTTACCAA AATGCCAATG
421 GAAATTACTT AGTATGTATA TAATGTATAA AGGTATGAAT ATCACAAACA GCAAATCGGC
481 TATTCCCAAG TTGAGAAACG GTATAATAGA TATATTCTA GATACCATTAA ATAACCTTAT
541 AAGCTTGACG TTTCCTATAA TGCCCTACTAA GAAAACCTAGA AGATACATAC ATACTAACGC
601 CATACTGAGAG TAACTACTCA TCGTATAACT ACTGTTGCTA ACAGTGACAC TGATGTTATA
661 ACTCATCTTT GATGTTGGTAT AAATGTATAA TAACTATATT ACACGTGTTAT TTTATTTCA
721 TTATATACTA TATAGTATTA AAAATTATAT TTGTATAATT ATATTATTAT ATTCACTGTA
781 GAAAGTAAAA TACTATAAT ATGTATCTCT TATTATAAC TTATTAGTAA AGTATGTACT
841 ATTCACTTAT ATTGTTTTAT AAAAGCTAAA TGCTACTAGA TTGATATAAA TGAATATGTA
901 ATAAATTAGT AATGTAGTAT ACTAATATTA ACTCACATTG GACTAATTAG CTATAAAAAC
961 CCGGGCTGCA GCCCGGGAAAG CTTACAAAAA TTAGACAAGA TTTGTTTCAG TATCATCACC
1021 GCTATCAAGA TTATTGAGGC GTTGGTATTT AGGTCCACGG CGACGGAGGG CCATGTTAGT
1081 GAGATGACTC GTAGGAGTCG CTGGGCCCTT ATTCTTTTC ATCGCCTTAT GTTCTTGTG
1141 CTCCATAGCC GATACGAGGG ACATATATT TATCATCTCC CTTGCCTGCA TTAGCTTTTC
1201 CTCATCGAAG TCATCGACTC CCGGGTCGCT ATCCCCACCA GCCGTTGAGC GGGCTCTTAG
1261 CGTCTGTTTC AAATTCTTAG TCCTCACAGG ATATAAGGCT TTCATTGGAT TTGACGTAA
1321 TCTAGATATA TAGCGATATG CCAGGAATGC TGCGACGATG CCAGCTATAA TTACAGTCC
1381 CACGGCCAAT GCTCCAAATG GGTTGTTAG AAATGATGAT ACGCCTGATA CTGTTGAGAT
1441 TACCGCACTC GCAGCCCCTA AGACCACTT GCGAAACCA GCCCCCACAT CCCGAGTCC
1501 CTGAAAGAAA TTTGCCATAC CACGCATGAT GACAAGGATTA TTATCCACTC TGACTATGCT
1561 GTCTATATCA TAAAATTCTA AGGCGTGGAG TTGGTTGCGG CGTTGAATCT CGCTGTAGTC
1621 CAAAAGGCCG GTATCTTCCA GCTCAGCTCG TGTATAAAACT TCGAGTGGGA GAAATTCA
1681 ATCCTCTAGG AGAGTAAGAT TTAATTACAC ATACGCACTT ATCAGTTCTA TCTCCGATAG
1741 CGGGACTTTA CGGACATACG CATAATCCTC AAAATATACA TAATCTGCC CAAACTTAAA
1801 ATACCGCTTA TTATTGACAG TGCAAGGCTC AATTAGTTT CGTTCCACGA GAAGTTCATT
1861 GTTTCCCT AGTTGTCCTT CTATGTATTC GGAGTCATTG AGGGCACGGA AGGAAACAAG
1921 AGGGCGGCTG TAACATGTCG TTGATGAACC CGTCACCCGC ATAGAATTCTT GGATATAGAC
1981 ATGTCCGCTA GAAATGTTAA CACATTGTGT TACGGCGACG GCATCTCCAA GTAGGCGC
2041 GGATACACGA CGTTCTAGGG CCATCGAGAC CACCCACCG GGATTGAGTT TTAGGGTCTC
2101 TGTCCACAGC ACATGTTCGC GGTTCTGAAG TGTACACCAG GCAGTGGCTA TCCGACTCAA
2161 CATTTCATT ACATGGGCTT GTATGTAGTC ATAGGCAAAT TGTAGCATTG CGAATTCCAC
2221 AGATGAGGTG GTCTTGAGGA GTGATGCATT GTTCACGGTT TCTATACCCCC CCTCTATTGT

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Fig. 5 (cont.)

2281 GCTGCGCCGC GACCTATGAT GTTGATTAGA TGGGACCGAT CTTCTAGTTC GTTGTACTGT
 2341 TTCCCCAGAT GGATTGAGGA GTGCACTGAG ATCTACCGTG CGATTTGAAC GTGCTAAC
 2401 ATTGATATAT AACTTTGCTA GTTCGTTGCT GATCATGGGA CGGAAAGCTA TTAGAAATCC
 2461 CCCACGGGCT AGGTAGGTCT CCAGGGTTCC AGTCTGAATA TGAGTTTAC TATATTACT
 2521 CTTATAAATC CGGTCTATGG CTTCGGCTGC CTCCCTGGTG GCACAGTCCC CCAAACGGAT
 2581 ACGATTGATT TCAAATTGTA AAGTATTGGA GATGAAAGTA GCGGATATGG TCTTGGCTGT
 2641 AAATCTATAG GAGCCCTGAT ATTCACTCGG TAGCATTCA TCTATTTCC TCCATTGGC
 2701 TAAGGTACAT ACCCGACCAG ACTTTGGGGT CCAGTTCCAG GCCACTGTCA CATCGGGAGT
 2761 TTCCAAAAAA TTGCGAGAAA CTGGTGCCCC CAGTTGTAAT CGCGTATCCA AGTCTATTGG
 2821 ATAGTATCCC TCGATTGTT GAAATCTGTC TGAAAGATAA CTAGTATGTT CTACATGGC
 2881 TCCATCCCTC AGCCCCAAGA ATGGAGACAT GTGAATCAGC TCACCAAGTGG AGATAGCAAA
 2941 TGAGTCATAT GGATATACAG ATCTTGCATC CACTTCCTCT ACGATGCAAT TTACAGAGGT
 3001 CCCAGAGTGG TGAAATCCAG CAGCACCGAT CTTTGTGTAT GTTTCATTGG TGGTGTGCCA
 3061 TCCACGGGAC TCTGGAGTGT TGAACCTGGA GGGTTTCAGA GGCAGTTCTC TGGGATCCTC
 3121 GTCTCGATCA AAGGCCGTAA ATTGATAATT GTTACGAACG TAATCAGCTT TCGAGAGGCA
 3181 CATACCCCGT CTATCTATGA GATCTGTAAT CTCTTGAACCT TTCACGGGAA CCCTGTCTGT
 3241 ATATCGGTTG GTTGTAAACGG CATAGGAACCT CCCAGACCAT ACCGTTGTCA TAATAATGTT
 3301 TTTATAGTAT ATATTTGCCT TGAATTATAA TGGCGCTATA TTTTCTTTAA ATATTACAGC
 3361 TATACCCCTCG GTAAAATTT TCCCTAGTT ATAATCTGGA CAGGCCGTG GTGGCTCTAA
 3421 ACGCACGACA GTAGATCCTG AAGGTGGTGG ACACATATAG AAAGTCGATG GTCCGTTAGC
 3481 CTCTATTGGA GACGCACCGA GAGCTCCCT CATATCCGAC ATATCGGTGG GATCTGTAGT
 3541 TGGTTTTGGT GGTGTACCCCC CTACCTCAGG GGTAGCTACA GTCCGGGGG GTTGTTCGA
 3601 TGTGCTTCCA GATTGACCCCT CTACTGGACG GGGACCGACT AAGAATAGTA CGATCCAGAT
 3661 AAATGAAACA TATCTAGCTA GTCTGGTTAA TCCCGACGAT CGTTACCAT GTCTGGAGCC
 3721 AGTCGCTGCA ATACCGAGTA GAGAAGGGAA AAAACATCTC TGTCGAAAT AGCCACTGTG
 3781 TCCCTGCCAA CGACTCCCTC GTGCCGCTT CCCAAGATCG CCACGAGTGG ACATTACGAT
 3841 ACAAACTTAA CGGATATCGC GATAATGAAA TAATTATGA TTATTCTCG CTTCAATT
 3901 AACACAACCC TCAAGAACCT TTGTATTTAT TTTCACTTTT TAAGTATAGA ATAAAGAAC
 3961 TCTAATTAAT TAAGCTACAA ATAGTTTCTG TTTCACCTTG TCTAATAACT ATTAATTA
 4021 CCCGGATCGA TCCCGATTTT TATGACTAGT TAATCAAATA AAAAGCATAAC AAGCTATTGC
 4081 T'CGCTATCG TTACAAAATG GCAGGAATT TGTGTAACCT AAGCCACATA CTTGCCAATG
 4141 AAAAAAATAG TAGAAAGGAT ACTATTTAA TGGGATTAGA TGTAAAGTT CCTTGGGATT
 4201 ATAGTAACTG GGCATCTGTT AACTTTACG ACGTTAGGTT AGATACTGAT GTTACAGATT
 4261 ATAATAATGT TACAATAAA TACATGACAG GATGTGATAT TTTTCTCAT ATAACCTTTG
 4321 GAATGCAAA TATGGATCAA TGTGATAGAT TTGAAAATT CAAAAGCAA ATAACGTGATC
 4381 AAGATTTACA GACTATTTCT ATAGTCTGTA AAGAAGAGAT GTGTTTCCCT CAGAGTAACG
 4441 CCTCTAAACA GTGGGGAGCG AAAGGATGCG CTGTAGTTAT GAAACTGGAG GTATCTGATG
 4501 AACCTAGAGC CCTAAGAAAT GTTCTGCTGA ATGCCGTACC CTGTTGAAG GACGTGTTG
 4561 GTGATATCAC ACTAGATAAT CCGTGGAAATC CTCACATAAC AGTAGGATAT GTTAAGGAGG
 4621 ACGATGTCGA AAACAAGAAA CGCCTAATGG AGTCATGTC CAAGTTAGG GGGCAAGAAA
 4681 TACAAGTTCT AGGATGGTAT TAATAAGTAT CTAAGTATTG GGTATAATT ATTAAATAGT
 4741 ATAATTATAA CAAATAATAA ATAACATGAT AACGGTTTTT ATTAGAATAA AATAGAGATA
 4801 ATATCATAAT GATATATAAT ACTTCATTAC CAGAAAATGAG TAATGGAAGA CTTATAATG
 4861 AACTGCATAA AGCTATAAGG TATAGAGATA TAAATTTAGT AAGGTATATA CTTAAAAAAT
 4921 GCAAATACAA TAACGTAAT ATACTATCAA CGTCTTGTG TTTAGCCGT AGTATTTCTG
 4981 ATATAGAAAT GGTAAAATTA TTACTAGAAC ACGGTGCCGA TATTTAAAA TGTAATTA
 5041 CTCCTCTCA TAAAGCTGCT AGTTAGATA ATACAGAAAT TGCTAAACTA CTAATAGATT
 5101 CTGGCGCTGA CATAGAACAG ATACATTCTG GAAATAGTCC GTTATATATT TCTGTATATA
 5161 GAAACAATAA GTCATTAAC AGATATTTAT TAAAAAAGG TGTTAATTGT AATAGATTCT
 5221 TTCTAAATTA TTACGATGTA CTGTATGATA AGATATCTGA TGATATGTAT AAAATATTTA
 5281 TAGATTTAA TATTGATCTT AATATACAAA CTAGAAATT TGAAACTCCG TTACATTACG
 5341 CTATAAAGTA TAAGAATATA GATTTAATT GGATATTGTT AGATAATAGT ATTAAAATAG
 5401 ATAAAAGTTT ATTTTGCTA AAACAGTATC TCATAAAGGC ACTTAAAAT AATTGTAGTT
 5461 ACGATATAAT AGCGTTACTT ATAAATCACG GAGTGCCTAT AACGAACAA GATGATTAG

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Fig. 5 (con. 4)

5521 GTAAAACCCC ATTACATCAT TCGGTAATT AAGAAGAAA AGATGAAACA GCACTTCTGT
5581 TAAATCTAGG AGCTGATATA AACGTAATAG ATGACTGTAT GGGCAGTCCC TTACATTACG
5641 CTGTTTCACG TAACGATATC GAAACAACAA AGACACTTT AGAAAGAGGA TCTAATGTTA
5701 ATGTGGTTAA TAATCATATA GATACCGTTC TAAATATAGC TGTTGCATCT AAAAACAAAAA
5761 CTATAGTAAA CTTATTACTG AAGTACGGTA CTGATACAAA GTTGGTAGGA TTAGATAAAC
5821 ATGTTATTCA CATAGCTATA GAAATGAAAG ATATTAATAT ACTGAATGCG ATCTTATTAT
5881 ATGGTTGCTA TGTAACGTC TATAATCATA AAGGTTTCAC TCCTCTATAC ATGGCAGTTA
5941 GTTCTATGAA AACAGAATT GTTAAACTCT TACTTGACCA CGGTGCTTAC GTAAATGCTA
6001 AAGCTAAGTT ATCTGGAAAT ACTCCTTTAC ATAAAGCTAT GTTATCTAAT AGTTTAATA
6061 ATATAAAATT ACTTTTATCT TATAACGCCG ACTATAATT TCTAAATAAT CACGGTAATA
6121 CGCCTCTAAC TTGTGTTAGC TTTTAGATG ACAAGATAGC TATTATGATA ATATCTAAA
6181 TGATGTTAGA AATATCTAAA AATCCTGAAA TAGCTAATT AGAAGGTTT ATAGTAAACA
6241 TGGAACATAT AACACAGTAAT AAAAGACTAC TATCTATAA AGAATCATGC GAAAAGAAC
6301 TAGATGTTAT AACACATATA AAGTTAAATT CTATATATT TTTAATATC TTTCTTGACA
6361 ATAACATAGA TCTTATGGTA AAGTTCGTAA CTAATCCTAG AGTTAATAAG ATACCTGCAT
6421 GTATACGTAT ATATAGGGAA TTAATACGGA AAAATAATC ATTAGCTTT CATAGACATC
6481 AGCTAATAGT TAAAGCTGTA AAAGAGAGTA AGAATCTAGG AATAATAGGT AGGTTACCTA
6541 TAGATATCAA ACATATAATA ATGGAACATAT TAAGTAATAA TGATTTACAT TCTGTTATCA
6601 CCAGCTGTTG TAACCCAGTA GTATAAAG

//

Fig. 6

POL/NEF epitopes

10	20	30	40	50	60	70	80	90	100	110
*	*	*	*	*	*	*	*	*	*	*
TTTTTTTCAT TATTTAGAAA TTATGCATT TAGATCTTA TAAGCGGCCG TGATTAACCA GTCATAAAA CCCGGGATCG ATTCTAGACT CGAGGGTACC GGATCTTAAT										
AAAAAAAGTA ATAATCTTT AATACGTAA ATCTAGAAAT ATTCCGCCGC ACTAATTGAT CAGTATTTT GGGCCCTAGC TAAGATCTGA GCTCCCATGG CCTAGAATTA										
120	130	140	150	160	170	180	190	200	210	220
*	*	*	*	*	*	*	*	*	*	*
TAATTAGTCA TCAGGCAGGG CGAGAACGAG ACTATCTGCT CGTTAATTAA TTAGGTCGAC GGATCCCCA ACAAAAACCA ATCAGCTATC GGGGTTAATT AATTAGITAT										
ATTAATCAGT AGTCCGTCCC GCTCTTGCTC TGATAGACGA GCAATTAAAT AATCCAGCTG CCTAGGGGT GTTTTTGAT TAGTCGATAG CCCCAATTAA TTAATCAATA										
230	240	250	260	270	280	290	300	310	320	330
*	*	*	*	*	*	*	*	*	*	*
TAGACAAGGT GAAAACGAAA CTATTTGAG CTTAATTAAAT TAGAGCTTCT TTATTCATAA CTTAAAAGT GAAAATAAAAT ACAAAAGGTT TTGAGGGTTG TGTTAAATTG										
ATCTGTTCCA CTTTGCTTT GATAAACATC GAATTAAATTA ATCTCGAAGA AATAAGATAT GAATTTTCA CTTTTATTTA TGTTTCCAAG AACTCCAAAC ACAATTAAAC										
H6 promoter										
340	350	360	370	380	390	400	410	420	430	440
*	*	*	*	*	*	*	*	*	*	*
AAAGCGAGAA ATAATCATAA ATTATTTCAT TATCGCGATA TCCGTTAAGT TTGTATCGTA ATG CCA CTA ACA GAA GAA GCA GAG CTA GAA CTG GCA GAA AAC										
TTTCGCTCTT TATTAGTATT TAATAAAAGTA ATAGCGCTAT AGGCAATTCA AACATAGCAT TAC GGT GAT TGT CTT CTT CGT CTC GAT CTT GAC CGT CTT TTG										
H6 promoter > Met Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn										
POL/NEF Epitopes										
450	460	470	480	490	500	510	520	530	540	550
*	*	*	*	*	*	*	*	*	*	*
AGA GAG ATT CTA AAA GAA CCA GTA CAT GGA GTG TAT TAT GAC CCA TCA AAA GAC TTA ATA GCA GAA ATA CAG AAG CAG GGG CAA GGC CAA										
TCT CTC TAA GAT TTT CTT GGT CAT GTA CCT CAC ATA ATA CTG GGT AGT TTT CTG AAT TAT CGT CTT TAT GTC TTC GTC CCC GTT CCG GTT										
Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln										
POL/NEF Epitopes										
560	570	580	590	600	610	620	630	640	650	660
*	*	*	*	*	*	*	*	*	*	*
TGG ACA TAT CAA ATT TAT CAA GAG CCA TTT AAA AAT CTG AAA ACA GGA ATG GAG TGG AGA TTT GAT TCT AGA TTA GCA TTT CAT CAC GTC										
ACC TGT ATA GTT TAA ATA GTT CTC GGT AAA TTT TTA GAC TTT TGT CCT TAC CTC ACC TCT AAA CTA AGA TCT AAT CGT AAA GTC GTG CAT										
Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Met Glu Trp Arg Phe Asp Ser Arg Leu Ala Phe His His Val										
POL/NEF Epitopes										
670	680	690	700	710	720	730	740	750	760	770
*	*	*	*	*	*	*	*	*	*	*
GCT AGA GAA TTA CAT CCT GAA TAT TTT AAA AAT TGT AAG CTT ATG GCA ATA TTC CAA AGT AGC ATG ACA AAA ATC TTA GAG CCT TTT AGA										
CGA TCT CTT AAT GTC GGA CTT ATA AAA TTT TTA ACA TTC GAA TAC CGT TAT AAG GTT TCA TCG TAC TGT TTT TAG AAT CTC GGA AAA TCT										
Ala Arg Glu Leu His Pro Glu Tyr Phe Lys Asn Cys Lys Leu Met Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg										
POL/NEF Epitopes										
780	790	800	810	820	830	840	850	860	870	880
*	*	*	*	*	*	*	*	*	*	*
AAA CAA AAT CCA GAC ATA GTT ATC TAT CAA TAC ATG GAT GAT TTG TAT GTC GGA TCT GAC TTA GAA ATA GGG CAG CAT AGA ACA AAA ATA										
TTT GTT TTA GGT CTG TAT CAA TAG ATA GTT ATG TAC CTA CTA AAC ATA CAT CCT AGA CTG AAT CTT TAT CCC GTC GTC TCT TGT TTT TAT										
Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile										
POL/NEF Epitopes										
POL/NEF Epitopes										

Fig. 6 (cont'd)

890	900	910	920	930	940	950	960	970		
*	*	*	*	*	*	*	*	*		
TAC AAA GCA GCT GTA GAT CTT TCT CAC TTT TTA AAA GAA AAA GGA GGT TTA GAA GGG CTA ATT CAT TCT CAA CGA AGA CAA GAT ATT CTT ATG TTT CGT CGA CAT CTA GAA AGA GTG AAA AAT TTT CTT TTT CCT CCA AAT CCT CCC GAT TAA GTA AGA GTT GCT TCT GTT CTA TAA GAA Tyr Lys Ala Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Arg Arg Gln Asp Ile Leu	POL/NEF Epitopes								>	
980	990	1000	1010	1020	1030	1040	1050	1060		
*	*	*	*	*	*	*	*	*		
GAT TTG TGG ATT TAT CAT ACA CAA GGA TAT TTT CCT GAT TGG CAG AAT TAC ACA CCA GGA CCA GGA GTC AGA TAC CCA TTA ACC TTT GGT CTA AAC ACC TAA ATA GTA TGT GTT CCT ATA AAA GGA CTA ACC GTC TTA ATG TGT GGT CCT CCT GGT CAG TCT ATG GGT AAT TGG AAA CCA Asp Leu Trp Ile Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly	POL/NEF Epitopes								>	
1070	1080	1090	1100	1110	1120	1130	1140	1150		
*	*	*	*	*	*	*	*	*		
TGG TGC TAC AAG CTA GTA CCA ATG ATT GAG ACT GTA CCA GTA AAA TTA AAG CCA GGA ATG GAT GGC CCA AAA GTT AAA CAA TGG CCA TTG ACC ACG ATG TTC GAT CAT GGT TAC TAA CTC TGA CAT GGT CAT TTT AAT TTC GGT CCT TAC CTA CCG GGT TTT CAA TTT GTT ACC GGT AAC Trp Cys Tyr Lys Leu Val Pro Met Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu	POL/NEF Epitopes								>	
1160	1170	1180	1190	1200	1210	1220	1230	1240		
*	*	*	*	*	*	*	*	*		
ACA GAA GAA AAA ATA AAA GCA TTA GTA GAA ATT TGT ACA GAG ATG GAA AAG GAA GGG AAA ATT TCA AAA ATT GGG CCT TAA TTTTTCT TGT CTT CTT TTT TAT TTT CGT AAT CAT CTT TAA ACA TGT CTC TAC CTT TTC CTT CCC TTT TAA AGT TTT TAA CCC GGA ATT AAAAAGA Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro	POL/NEF Epitopes								>	
1250	1260	1270	1280	1290	1300	1310	1320	1330	1340	1350
*	*	*	*	*	*	*	*	*	*	*
GCAGCCCCGGG GGATCCTTT TATAGCTAAT TAGTCACGTA CCTTGAGAG TACCACTTCA GCTACCTCTT TTGTGTCTCA GAGTAACCTT CTAAATCAA TTCCAAAACA CGTCGGGGCCC CCTAGAAAAA ATATCGATTA ATCAGTGCAT GGAAACTCTC ATGGTGAGT CGATGGAGAA AACACAGAGT CTCATTGAAA GAAATTAGTT AAGGTTTGT										

Fig. 7

gag (+ pro) and gp120 (+ transmembrane)

FEATURES	From	To/Span	Description									
frag	1	56	C3 flanking arm									
frag	162	76 (C)	HIV1 (IIIB) env transmembrane region									
frag	1728	163 (C)	HIV1 (MN) gp120 gene									
frag	1853	1729 (C)	vaccinia K6 promoter									
frag	1925	1983	vaccinia I3L promoter									
frag	1984	3746	HIV1 (IIIB) gag/pro gene									
frag	3753	3808	C3 flanking arm									
	10	20	30	40	50	60	70	80	90	100	110	120
TAATGTAGTATACTAATATTAACTCACATTGACTAATTAGCTATAAAAACCCGGGATCGATTCTAGAATAAAAATTATCCCCTGCCTAACACTCTATTCACTACAGAGAGTACAGCAAAAC												
ATTACATCATATGATTATAATTGAGTGAACTGATTAATCGATATTGGCCCTAGCTAAGATCTTAACTTAAAGGGGAGGTTAGAGATAAGTGATGTCATGTCATGCTCGTTTTTG												
<u>C3 FLANKING ARM</u>	<u>></u>											
	130	140	150	160	170	180	190	200	210	220	230	240
TATTCTTAAACCTACCAAGCCTCTACTACATTATGAATAATCTTTTCTCTGACCCTCTCTTGCCTTGGGTGCTACTCCTAACATGGTCAATTGTTACTACTTATA												
ATAAGAATTGGATGGTCCGGAGGATGATGAGTAACTTATTAGAAAAAGAGAGACGGTGGAGAAAGAGAACGGACACCCACCGATGAGGATTACCAAGTTAACATGATGAAAT												
<u>HIV1 (IIIB) ENV TRANSMEMBRANE REGION</u>	<u><</u>											
	190	200	210	220	230	240						
G Q R V R N V V S L V A F V												
	250	260	270	280	290	300	310	320	330	340	350	360
TTTATATAATTCACTTCTCAATTGTCCTCATATCTCCTCCAGGTCTGAAGATCTGGTGTGCTTACACCATCTCTGTTAATAGTAGCCCCGTAAATTATTAA												
AAATATTAATTAAAGTAAGAGGTTAACAGGGAGTATAGAGGGAGGCTCAGACTCTAGGCCACAGGCACAGGAATGGTGTAGAGAACAAATTATCATCGGGACATTAA												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											
	370	380	390	400	410	420	430	440	450	460	470	480
TGATGAACATCTAATTGTCCTCAATGGGAGGGCATATATTGCTTTCTACTTCTGCCACATGTTATAATTGTTTATTGCTATTGAAGTGTGATATTGTTATTGACCCGT												
ACTACTGTAGATTAAACAGGAATTACCCCTCCCGTATATAACGAAAAGGATGAGGACGGTGTACAAATATTAAACAAATAACGTAACCTCACACTATAACAAACTGGGACA												
S S C R I Q G E I P P A Y I A K G V E Q W M N I I Q K I K C Q L T I N H N N S G T												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											
	490	500	510	520	530	540	550	560	570	580	590	600
AGTATTATCCAAGTATTATTACCATCCAAGTACTATTAAACAGTGGTGTGATTAACAGTAGAGAAATTCCCTCCACAATTAAAACGTGCATTACAATTCTGGTCCCTCTGA												
TCATAATAAGGTTATAATAATGGTAAGGGTCTGATAATTGTCAACCTACTTAATGTCTTAAAGGGAGGTGTAAATTGACACGTAATGTAAAGACCCAGGGAGGACT												
T N N W T N N G N W T S N F L P S S N C Y F E G G C N F S H M V I E P D G G S												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											
	610	620	630	640	650	660	670	680	690	700	710	720
GGATTGATTAAAGACTATTGTTATTCTAAATTGCTCTTAAATTGCTAACTATCTGCTTAAAGGTCTATTCCATTGCTCTACTAATGTTACAATGTGCTTGTCTTATAGTT												
CCTAACTAATTCTGATAACAAAATAAGAATTAAACAAAATAACGATTGAGACAGAAATTCTACAGTAAGGTAACAGAGATGATTACAATGTTACACGAACAGAAATCAAGG												
S Q N F V I T K N K F Q E K L K S V I O R L T D N W K A R S I N C H A Q R I T G												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											
	730	740	750	760	770	780	790	800	810	820	830	840
TATTATATTTTGTGTATAAAAATGCTCTCCCTGCTCTATATGTAATCCCTTTCTTTATTGTTAGTTGGGTCTGTACAATTATTTGACAGATTCACTGAGTACTATGATGGT												
ATAATATAAAAACACATATTACCGAGAGGGACCGAGTATACATAGGAAAAAGAAAATAACATCAACCCAGAACATGTAATTAACATGCTAAAGTCAAGTCTACATGATACTACCA												
I I N K T T Y F A R G P G I H I R K R N Y N P R T C N I Q V S E N L H V I I T												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											
	850	860	870	880	890	900	910	920	930	940	950	960
TTTAGCATTATCATGAAATTCTCAGATCTAACTACTACCTCTCTGCTAGACTGCCATTAAACAGCAGTTGAGTTGATACACTGGCTAACCTCATGTCATATTGACTGTGCT												
AAATCGTAATGTAACCTTAAGAGCTAGATTAATGATGGAGAAAGAACGATCTGACGGTAATTGTCATGTCACACTCAACTATGATGACCCGATTAAGGTACACATGTAACATGACAGCA												
K A N D N F H E S R I V V E E A L S G N L L L Q T S V V P R I G H T C Q V T S												
<u>HIV1 (MN) GP120 GENE</u>	<u><</u>											

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Fig. 7 (cont'd)

970 980 990 1000 1010 1020 1030 1040 1050 1060 1070 1080
 GACATTTTACATGATCCTTCCACTGAACCTTTATCGTACACTTAAAGTCGAAACCCAGCCGGGCACAATAGTGATGGCAATTGGCTCAAGGATATCTTGGACAAGCTG
 CTGAAAAATGTACTAGGAAAAGGTACTGAAAAATAGCAATGTAACTTACCGTTTGGTCCGGCCCCGTATCACATACCCCTAACCGAGTTCTATAGAAACCTGTCGAAC
 V N K C S G K G S F K K D N C K L I A F G A P A C Y H I P I P E F S I K P C A Q
 < HIV1 (MN) GP120 GENE

1090 1100 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
 TGTAATGACTGAGGTATTACAACCTATACTGGTACTATCATTATTTATGATACTATCAAGTTAAGAAGTGCAATTCTCTGATCTTATCTTATGCTTGT
 ACATTACTGACTCCATAATGTGAATAGTGGATATCGACCATGATAGTAATAAAACTATGATATAGTCAATATTCTCACGTATAAGAAAGCAGTAGAATAGAGAATACGAAC
 T I V S T N C S I L R Y S T S D N N I S V I D L K Y L L A Y E K O M K D R I S T
 < HIV1 (MN) GP120 GENE

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320
 GGTGATATTGAAAGAGCAGTTTCATTCTCCTCCCTTATTGTTCCCTCGCTTACTTACTATTGTTATTAGCAGTACTATTGGTATTAGTAGTATTCTCAAATCAGTCAATTAA
 CCACTATACTTCTCGTCAAAAGTAAGAGGAGGGAAATAACAGGGAGCGATAATGATAACATAATCGTATGATAACCATATCATAGTAGGAGTTAGTCACGTAAATT
 T I N F S C N K M E G G K I T G E S N S N N A T S N N T N T N R L D T C N L
 < HIV1 (MN) GP120 GENE

1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430 1440
 AGAACACAGAGTGGGTTAATTACACATGGCTTGTAGGCTTAACTGTTACATGCTTACCATGTTACATGCTGTTACCATGTTAAACATGTTAAATTCTGTCAC
 TCATTGTCCTACCCCAATTAAATGTGACCGAAATCGGAAACTAGGGTATTGACTAATAGGAGTAGCAGACAAGATGGTACAATAAAAGGTGACAATTAAAGACAGT
 T V C L P T L K V C P K L S Q D W L S I D E H M O E V M N N K W M N F N E T V
 < HIV1 (MN) GP120 GENE

1450 1460 1470 1480 1490 1500 1510 1520 1530 1540 1550 1560
 ATTACCAATTCTACTCTGTGGGTTGGGTCTGTGGTACACAGGCAATGTGACCTCTGATCATGCTTACATGCTTACATGCTGATGACACAAAATAGAGTGGTGGT
 TAAATGTTAAAGATGAAGAACCCCAACCCCAGACACCCATGTGTCGTACACACGGGTTGTAATACATGGAGACATAGTACAGAAATCGTAGACTACGTGTTATCTACAC
 N V L E V E Q P N P D T P V C A H T A W V N H V E T D Y A K A D S A C F L T T T
 < HIV1 (MN) GP120 GENE

1570 1580 1590 1600 1610 1620 1630 1640 1650 1660 1670 1680
 TGCCTTCCACACAGGTACCCATAATAGACTGTGACCCACAATTCTGTAGCACTACAGATCATCACACATCCAAGGAGCATGGTCCCCATCTCCACCAAGTG
 ACGAAGAAAGGTGTGTCATGGGTATTATGTGACACTGGGTAAAGGACATCGTAGTGTCTAGTTAGGGTCTCTGTACACGGGTTAGAGGTTGGGTAGAGGTTGTCAC
 A E K W V P V G Y Y V T V W L K E T A S C I M H G L L H T G W R W G W R W L H
 < HIV1 (MN) GP120 GENE

1690 1700 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
 CTGATTTCTCCTCACTCTCATTGCCACTGTCTCTGCTCTTCATATACGATACAAACTTAACGATATCGCGATAATGAAATAATTATGATTATTCTCGTTCAATTAAACAC
 GACTAAAGAGGAAGTGAAGAGTAACGGTACAGAAGCAGAGAAAGTATATGCTATGTTGAATTGCGTAGCGCTTACCTTAAACTAATAAAAGCAGAAAGTTAAATTG
 Q Y K E K V R M A V T K Q E K M
 < HIV1 (MN) GP120 GENE

VACCINIA H6 PROMOTER

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900 1910 1920
 AACCTCAAGAACCTTGATTATTTCACTTTAAGTATAGAATAAGAAGCTAATTAAGCTACAAATAGTTCGTTTACCTTGCTAATAACTAATAAAACCCCG
 TTGGGAGTTCTGGAAACATAATAAAAGTAAAAATCATCTTCTCGAGATTAATTCGATGTTATCAAGCAAAGTGAACAGATTATGATTAATTAATTGGGCC
 < VACCINIA H6 PROMOTER

1930 1940 1950 1960 1970 1980 1990 2000 2010 2020 2030 2040
 ATCTTGAGATAAAAGTAAAATATATCATTATATTACAAAGTACAATTATTAAGGTTAATCATGGGTCGAGAGCGCTAGTAAAGGGGGGAGAATTAGATCGATGGAAAAATT
 TAGACTCTATTCACTTTATATATGATAATATAAGTGTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTTATGTT
 VACCINIA 13L PROMOTER

> M G A R A S V L S G G E L D R W E K I

< HIV1 (IIB) GAG/PRO GENE

2050 2060 2070 2080 2090 2100 2110 2120 2130 2140 2150 2160
 CGGTTAAGGCCAGGGGAAAGAAAAATAAAATTAAACATAGTATGGCAAGCAGGGAGCTAGAACGATTCGAGTAACTCGCTGCTGTTAGAACATCAGAAGGCTGAGACAA
 GCCAATTCCGGTCCCCCTTCTTTTATTTAATTGTATATCATACCGTTCTCGCTCGATCTGCTAAGCGTCAATTAGGACCGGACAATCTTGTAGTCTCCGACATCTGTT
 R L R P G G K K K Y K L K H I V W A S R E L E R F A V N P G L L E T S E G C R Q
 < HIV1 (IIB) GAG/PRO GENE

2170 2180 2190 2200 2210 2220 2230 2240 2250 2260 2270 2280

Fig. 7 (cont')

ATACTGGGACAGCTACAACCATCCCTCAGACAGGATCAGAAGAACTTAGATCATTATAATAACAGTACCAACCCCTATTGTGTGATCAAAGGATAGAGATAAAAGACACCAAGGAA
 TATGACCCCTGTGATGGTAGGGAAGTCGTCCTAGTCTTCTGAATCTAGTAATAATTATGTACATCGTTGGAGATAACACAGCTAGTTCTATCTATTTCTGTGGTCCCT
 I L G O L Q P S L Q T G S E E L R S L Y H T V A T L Y C V H Q R I E I K D T K E >
 HIV1 (IIIB) GAG/PRO GENE

2290 2300 2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
 GCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAGCAACAGCAGCAGCTGACACAGGACACCAATCAGGTACCCAAATTACCCATAGTGAGAACATCCAG
 CGAAATCTGTCTATCTCTCTCGTTTGTCTTCTGTCGTCGTCGACTGTGTCCTGTGTCGTTAGTCCAGTCGGTTAAAGGGATATCACGTCCTGTAGGTC
 A L D K I E E E Q N K S K K A Q Q A A A D T G H S N Q V S Q N Y P I V Q N I Q >
 HIV1 (IIIB) GAG/PRO GENE

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500 2510 2520
 GGGCAATGGTACATCAGGCCATATCACCTAGAATTAAATGCTGGTAAAGTAGTAGAAGAGAAGGCTTCAGCCCAGAAGTGTATACCCATGTTTCAGCATTATCAGAAGGAGCC
 CCCGTTACCGTAGTCGGTAAAGTGGATCTGAAATTACGTACCCATTTCATCATCTCTCCGAAAGTCGGGCTTCACTATGGTACAAAAGTCGTAATAGTCCTCTCGG
 G O M V H Q A I S P R T L N A W V K V V E E K A F S P E V I P M F S A L S E G A >
 HIV1 (IIIB) GAG/PRO GENE

2530 2540 2550 2560 2570 2580 2590 2600 2610 2620 2630 2640
 ACCCCACAAGATTAAACACCATGCTAAACACAGTGGGGGACATCAAGCAGCCATGCAAATGTTAAAGAGACCATCAATGAGGAAGCTCGAGAATGGATAGTGATCCAGTGAT
 TGGGGTGTCTAAATTGTTGGTACGATTGTGTCACCCCCCTGTAGTTCTGTCGTCAGTTACAAATTCTCTGGTAGTTACTCTTCGACGTCTACCCATCTCACGTCAGTCAGT
 T P Q D L N T M L N T V G G H Q A A M Q M L K E T I N E E A A E W D R V H P V H >
 HIV1 (IIIB) GAG/PRO GENE

2650 2660 2670 2680 2690 2700 2710 2720 2730 2740 2750 2760
 GCAGGGCTATTGACCCAGGCCAGATGAGAGAACCAAGGGGAAGTGTACATAGCAGGAACACTACTGTACCCCTCAGGAACAAATAGGATGGATGACAATTCACCCATCCAGTAGGA
 CGTCCCGGATAACGTGGTCCGGTACTCTCTGGTCCCTACTGTATCGTCCTGGTAGTACGGAAAGTCCTGTTATCTCACCTACTGTATTAGGTGGATAGGGTCATCC
 A G P I A P G Q M R E P R G S D I A G T T S T L Q E Q I G W M T N N P I P V G >
 HIV1 (IIIB) GAG/PRO GENE

2770 2780 2790 2800 2810 2820 2830 2840 2850 2860 2870 2880
 GAAATTATAAAAGATGGATAATCTGGGATAAATAAAATGTAAGAATGTATAGCCCTACCGCATCTGGACATAAGAACAGGACAAAAGAACCTTAGAGACTATGTAGACCCG
 CTTTAAATATTTCTACCTATTAGGCCCTAATTATTTATCATCTACATCGGGATGGCTGAAGACCTGTATTCTGGTTCTGGAAATCTGTACATACATCTGGCC
 E I Y K R W I I L G L N K I V R H Y S P T S I L D I R Q G P K E P F R D Y V D R >
 HIV1 (IIIB) GAG/PRO GENE

2890 2900 2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
 TTCTATAAAACTCTAAGAGCCGAGCAAGCTTACAGGAGGTTAAAAAATTGGTACAGAGAACCTTGGTCCAAATGGCAACCCAGATTGAAGACTATTTAAAGCATTGGGACCA
 AAGATATTGAGATTCTCGGCTCTGGAAAGTGTCTCCATTTCACCTACTGTCTGGAAACACCAGTTACGGCTGGCTAACATCTGTATAAAATTCTGTAACCCCTGGT
 F Y K T L R A E Q A S Q E V K N W M T E T L L V O N A N P D C K T I L K A L G P >
 HIV1 (IIIB) GAG/PRO GENE

3010 3020 3030 3040 3050 3060 3070 3080 3090 3100 3110 3120
 GCGCCTACACTAGAAGAAAATGATGACAGCATGTCAGGGAGTAGGGAGGCCATAAGGCAAGAGCTTGGCTGAAGCAATGAGCCAACTAACAAATCGCTACCCATAATGATGCG
 CGCCGATGTCTCTTACTACTGTGTCAGTCCCTCATCTCCCTGGGGTATTCCGTCAAAACCGACTCGGTTACGGCTATTGTTAGTCGATGGTATTACTACGTC
 A A T L E E M M T A C Q G V G G P G H K A R V L A E A M S Q V T N S A T I M M Q >
 HIV1 (IIIB) GAG/PRO GENE

3130 3140 3150 3160 3170 3180 3190 3200 3210 3220 3230 3240
 AGAGGCAATTAGGAACCAAGAAAGATTGTTAAGTGTCTCAATTGTTGGCAAGAGGGACACAGCCAAAATTGGCAGGGCCCTAGGGAAAAGGGCTGGAAATGTTGGAAAGGAA
 TCTCGTTAAATCCTGGTTCTCTAACAAATTCAACAAAGTTAACACCGTTCTCCGTTGTCGGTCTTAAACGTCCTGGGATCTTCCCGACACCTTACACCTTCCCT
 R G N F R N Q R K I V K C F N C G K E G H T A R N C R A P R K K G C W K C G K E >
 HIV1 (IIIB) GAG/PRO GENE

3250 3260 3270 3280 3290 3300 3310 3320 3330 3340 3350 3360
 GGACACCAATGAAGATTGACTGAGAGACAGGCTAATTGGGAAGATCTGGCTTCTACAGGGAAGGCCAGGGAAATTCTCAGAGCAGACAGGCCAACAGCCCCACCA
 CCTGTGGTTACTCTAACATGACTCTGTGTCGATTAAAATCCCTCTAGACGGGAAGGATGTCCTCCGGTCCCTAAAGAAGTCGTCCTGGTCTCGGTGTCGGGTGG
 G H Q M K D C T E R Q A N F L G K I W P S Y K G R P G N F L Q S R P E P T A P P >
 HIV1 (IIIB) GAG/PRO GENE

Fig. 7 (cont'd)

3370 3380 3390 3400 3410 3420 3430 3440 3450 3460 3470 3480
 AAAGAGAGCTTCAGGCTGGGTAGAGACAACAACTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACACTGTATCCCTTAACCTTCACTCTGGCAACGACCCCTCGTCA
 TTCTCTCGAAGTCCAGACCCCATCTCTGTTGAGGGGGAGCTTCGCTCTCGCTATCTGTTCCCTGACATAGGAATTGAAGGGAGTCACTGAGAAACCGTTGCTGGGAGCAGT
 E E S F R S G V E T T T P P Q K Q E P I D K E L Y P L T S L R S L F G N O P S S
 P Q I T L W Q R P L V
HIV1 (IIIB) GAG/PRO GENE

3490 3500 3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
 AATAAGATAGGGGGCAACTAAAGGAAGCTCTATTAGATAACAGGAGCAGATGATACAGATTAGAAGAAATGAGTTGCCAGGAAGATGGAACAAAATGATAGGGGAATTGGAG
 TTATTTCTATCCCCCGTTGATTTCTCGAGATAATCTATGTCTCGTACTATGTCTAAATCTTCTACTCAAACGTCCTCTACCTTGGTTTACTATCCCCCTAACCTC
 Q I K I G G Q L K E A L L D T G A D D T V L E E M S L P G R W K P K H I G G I G
HIV1 (IIIB) GAG/PRO GENE

3610 3620 3630 3640 3650 3660 3670 3680 3690 3700 3710 3720
 TTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGGACATAAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCACATAATTGGAAGAAATCTGGTGA
 CTC
 AAAATAGTTCTTCTGTCATACTAGTCTATGAGTATCTTAGACACCTGTATTTCGATATCCATGTCTATAATCATCTGGATGTGGACAGTTGATTAACCTCTTAACTGAG
 F I K V R Q Y D Q I L I E I C G H K A I G T V L V G P T P V N I I G R N L T
HIV1 (IIIB) GAG/PRO GENE

3730 3740 3750 3760 3770 3780 3790 3800
 GATGGTTGCACTTTAAATTAAACCCGGGGATCCGATTTTATGACTGTTAATCAAATAAAAGCATACAAGCTATTGCTTC
 CTAACCAACGTGAAATTAAAAATTGGCCCCCTAGGGCTAAAATCTGATCAATTAGTTATTTCTGATGTTGATAACGAAG
 I G C T L N F < C3 FLANKING ARM >
HIV1 (IIIB) GAG/PRO

vcp1452

Fig. 8

K3L E3L in C6

* 10 20 30 40 50 60 70 80 90 100 110

GAGCTCGCGG CCCCTATCA AAAGTCTAA TGAGTTAGT GTAGATAGTA TAGATATTAC TACAAGGT TTCAATTTC CTATCAATTCA TAAAGTAGAT GATATTATA
CTCGAGGCC GGCGGATAGT TTTCAGAATT ACTCAATCCA CATCTATCAT ATCTATAATG ATGTTCCAT AAGTATAAG GATAGTAAAG ATTTCATCTA CTATAATT

* 120 130 140 150 160 170 180 190 200 210 220

ACTCAAAGAT GATGATAGTA GATAATAGAT ACCGTCATAT AATGACTGCA AATTGGACG GTTCACATT TAATCATCAC CGGTICATAA GTTCAACTG CATAGATCAA
TGAGTTCTA CTACTATCAT CTATTATCTA TGCGAGTATA TTACTGAGT TTAAACCTGC CAAGTGTAA ATTAGTAGTG CGCAAGTATT CAAAGTGAC GTATCTAGTT

* 230 240 250 260 270 280 290 300 310 320 330

AATCTCACTA AAAAGATAGC CGATGTATTG GAGAGAGATT GGACATCAA CTACGCTAA GAAATTACAG TTATAAATAA TACATAATGG ATTTGTTAT CATCACTT
TTAGAGTGT TTTCTATCG GCTACATAAA CCTCTCTAA CCTGTAGATT GATGCGATT CTAAATGTC AAATTTATT ATGTATTAC TAAAACAATA GTAGTCATA

* 340 350 360 370 380 390 400 410 420 430 440

ATTTAACATA AGTACAATAA AAAGTATTA AAATAAATAC TTACTTACGA AAAATGACT AATTAGCTAT AAAACCCAG ATCTCTCGAG GTCGACGGTA TCGATAAGCT
TAAATTGTAT TCATGTTATT TTTCAATAATT TATTTTATG AATGAATGCT TTTTACTGA TAAATCGATA TTTTGGGT TAGAGAGCTC CAGCTGCCAT AGCTATTGCA

* 450 460 470 480 490 500 510 520 530

TGATATCGAA TTCATAAAA TT A TTG ATG TCT ACA CAT CCT TTT GTA ATT GAC ATC TAT ATA TCC TTT TGT ATA ATC AAC TCT AAT CAC TTT
ACTATAGCTT AAGTATTTT AA T AAC TAC AGA TGT GTA GGA AAA CAT TAA CTG TAG ATA TAT AGG AAA ACA TAT TAG TTG AGA TTA GTG AAA
<Q H R C M R K Y N V D I Y G K T Y D V R I V -K

-----K3L-----

* 540 550 560 570 580 590 600 610 620

AAC TTT TAC AGT TTT CCC TAC CAG TTT ATC CCT ATA TTC AAC ATA TCT ATC CAT ATG CAT CTT AAC ACT CTC TGC CAA GAT AGC TTC AGA
TTG AAA ATG TCA AAA GGG ATG GTC AAA TAG GGA TAT AAG TTG TAT AGA TAG GTA TAC GTA GAA TTG TGA GAG AGC GTT CTA TCG AAG TCT
<V K V T K G V L K D R Y E V Y R D M H M K V S E A L I A E S

-----K3L-----

* 630 640 650 660 670 680 690 700 710

GTG AGG ATA GTC AAA AAG ATA AAT GTA TAG AGC ATA ATC CTT CTC GTA TAC TCT GCC CTT TAT TAC ATC GCC CGC ATT GGG CAA CGA ATA
CAC TCC TAT CAG TTT TTC TAT TTA CAT ATC TCG TAT TAG GAA GAG CAT ATG AGA CGG GAA ATA ATG TAG CGG GCG TAA CCC GTT GCT TAT
<H P Y D F L Y I Y L A Y D K E Y V R G K I V D G A N P L S Y

-----K3L-----

* 720 730 740 750 760 770 780 790 800 810

ACA AAA TGC AAG CAT ACG ATACAAACTT AACGGATATC GCGATAATGA AATAATTAT GATTATTCT CGCTTCAAT TAAACACAAAC CCTCAAGAAC
TGT TTT ACG TTC GTA TGC TATGTTGAA TTGCGTATAG CGCTTACTT TTATTAATA CTAATAAGA GCGAAAGTTA ATTGTGTT GGAGTTCTG
<C F A L M

-----K3L-----

* 820 830 840 850 860 870 880 890 900 910 920

CTTTGTATTT ATTTCACTT TTAAGTATA GAATAAGAA AGCTTAATT ATTAAATGAA CAGATTGTT CGTTTCCCC TTGGCGTATC ACTAATTAAT TAACCCGGC
GAAACATAAA TAAAGTGA AAATTCAAT CTTATTTCTT TCGAGATTAA TTAATTACTT GTCTAACAAA GCAGAAGGGG AACCGCATAG TGATTAATTA ATTGGGGCCG

* 930 940 950 960 970 980 990 1000 1010 1020 1030

TGCAGCTCGA GGAATTCAAC TATATCGACA TATTCATTT GTATACACAT AACCTTAAT AACGTAAT GTATAGGAAG AGATGTAACG GGAACAGGGT TTGTTGATT
ACGTCGAGCT CCTTAAGTT ATATAGCTGT ATAAAGTAA CATATGTGTA TTGTTAATGA TTGCGATCTA CATATCCTTC TCTACATTGC CCTTGTCACA AACAACTAAC

* 1040 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140

GCAAACTATT CTAATACATA ATTCTTCTGT TAATACGCT TGCACGTAAT CTATTATAGA TGCAAGATA TCTATATAAT TATTTGAA GATGATGTTA ACTATGTGAT
CGTTGATAA GATTATGTAT TAAGAAGACA ATTATGCGA ACGTGCATTA GATAATATCT ACGGTTCTAT AGATATATTA ATAAACATT CTACTACAT TGATACACTA

Fig. 8 (cont'd)

1150	1160	1170	1180	1190	1200	1210	1220	1230	1240	1250
CTATATAAGT AGTGTAAATA TTCACTGATT TCGATATATG TTCCAACCTCT GTCTTTGTGA TGTCAGTTT CGTAATATCT ATAGCATCCT CAAAAAATAT ATTGCGATAT GATATATTCA TCACATTATT AAGTACATAA AGCTATATAC AAGGTGAGA CAGAACACT ACAGATCAA GCATTATAGA TATCGTAGGA GTTTTTATA TAAGCGATA										
1260	1270	1280	1290	1300	1310	1320	1330	1340	1350	1360
ATCCCCAAGT CTTCAGTTCT ATCTTCTAAA AAATCTCAA CGTATGGAA ATAATAATCT ATTTTACCTC TTCTGATATC ATTAAATGATA TAGTTTTGAG CACTATCTC TAAGGGTCA GAAGTCAGA TAGAAGATT TTTAGAGTT GCATACCTA TATTATTAGA TAAATGGAG AAGACTATAG TAATTACTAT ATCAAAAATC GTGATAGAAG										
1370	1380	1390	1400	1410	1420	1430	1440	1450	1460	1470
TGCAATTGTA TTCTTATTCA CTATATCTAA GAAACGGATA GCGTCCCTAG GACGAACCTAC TGCCATTAA ATCTCTTAA TAGCTTCTGG ACATAATTCA TCTATTATAC ACAGTTAACT AAGAATAAGT GATATAGATT CTTGCCTAT CCCAGGGATC CTGCTTGATG ACGGTAATTA TAGAGATAAT ATCGAAGACC TGTTAAAGT AGATAATATG										
1480	1490	1500	1510	1520	1530	1540	1550	1560	1570	1580
CAGAATTAAT GGAACTATT CCGTATCTAT CTAACATAGT TTTAGAAAG TCAGAATCTA AGACCTGATG TTCATATATA GGTCATACA TGAAATGATC TCTATTGATG GTCTTAATTA CCCTTGATAA GGCATAGATA GATTGTATCA AAATCTTTC AGTCTTAGAT TCTGGACTAC AAGTATATAA CCAAGTATGT ACTTTACTAG AGATAACTAC										
1590	1600	1610	1620	1630	1640	1650	1660	1670	1680	1690
ATAGTGACTA TTTCATTCTC TGAAATTTGG TAACCTCATC TATATATGCT TTCTTGTTG ATGAAGGATA GAATATACTC AATAGAATT GTACCAACAA ACTGTTCTCT TATCACTGAT AAAGTAAGAG ACTTTAACC ATTGAGTAAG ATATATACGA AGGAACAAAC TACTTCCTAT CTTATATGAG TTATCTAAA CATGGTTGTT TGACAAGAGA										
1700	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800
TATGAATCGT ATATCATCAT CTGAATAAT CATGTAAGGC ATACATTAA CAATTAGAGA TTGTCCTCC GTTATCAATA TACTATTCTT GTGATAATTG ATGTGTGAGG ATCTTAGCA TATAGTAGTA GACTTTATTA GTACATTCCG TATGTAATT GTTAATCTCT GAACAGAGGA CAATAGTTAT ATGATAAGAA CACTATTTAA TACACACTCC										
1810	1820	1830	1840	1850	1860	1870	1880	1890	1900	1910
CAAATTTGTC CACGTTCTT AATTTGTTA TAGTAGATAT CAAATCCAAT GGAGCTACAG TTCTTGCTT AAACAGATAT AGTTTTCTG GAACAAATTC TACAACATTA GTTAACACAG GTGCAAGAAA TAAACAAAT ATCATCTATA GTTTAGGTTA CCTCGATGTC AAGAACCGAA TTTGCTATA TCAAAAGAC CTTGTTAAG ATGTTGAAT										
1920	1930	1940	1950	1960	1970	1980	1990	2000	2010	2020
TTATAAAGGA CTTGGGTAG ATAAGTGGGA TGAAATCCTA TTTTAATTAA TGCTATCGCA TTGTCCTCGT GCAAATATCC AAACGCTTT GTGATAGTAT GGCATTCTT AATATTCCT GAAACCCATC TATTCACCT ACTTTAGGAT AAAATTAATT AGCATAGCGT AACAGGAGCA CGTTTATAGG TTGCGAAAA CACTATCTATA CCGTAAGTAA										
2030	2040	2050	2060	2070	2080	2090	2100	2110	2120	2130
GTCTAGAAC GCTCTACGAA TATCTGTGAC AGATATCATC TTTAGAGAAT ATACTAGTCG CGTTAATAGT ACTACAATT GTATTTTA ATCTATCTCA ATAAAAAAAT CAGATCTTG CGAGATGCTT ATAGACACTG TCTATAGTAG AAATCTCTTA TATGATCAGC GCAATTATCA TGATGTTAA CATAAAAT TAGATAGAGT TATTTTTTA										
2140	2150	2160	2170	2180	2190	2200	2210	2220	2230	*
TAATATGTAT GATTCAATGT ATAACAAAC TACTAACTGT TATTGATAAC TAGAATCA GAA TCT AAT GAT GAC GTA ACC AAG AAG TTT ATC TAC TGC CAA ATTATACATA CTAAGTTACA TATTGATTG ATGATTGACA ATAACATTATG ATCTTAGT CTT AGA TTA CTA CTG CAT TGG TTC TTC AAA TAG ATG AGC GTT <F R I I V Y G L L K D V A L -----E3L-----										
2240	2250	2260	2270	2280	2290	2300	2310	2320	*	*
TTT AGC TGC ATT ATT TTT AGC ATC TCG TTT AGA TTT TCC ATC TGC CTT ATC GAA TAC TCT TCC GTC GAT GTC TAC ACA GGC ATA AAA TGT AAA TCG ACG TAA TAA AAA TCG TAG AGC AAA TCT AAA AGG TAG ACG GAA TAG CTT ATG AGA AGG CAG CTA CAG ATG TGT CCG TAT TTT ACA <K A A N N K A D R K S K G D A K D F V R G D I O V C A Y F T -----E3L-----										

. 21/22

Fig. 8 (cont'd.)

2330 * 2340 * 2350 * 2360 * 2370 * 2380 * 2390 * 2400 * 2410 *
 AGG AGA GTT ACT AGG CCC AAC TGA TTC AAT ACG AAA AGA CCA ATC TCT CTT AGT TAT TTG GCA GTA CTC ATT AAT AAT GGT GAC AGG GTT
 TCC TCT CAA TGA TCC GGG TTG ACT AAG TTA-TGC TTT TCT GGT TAG AGA GAA TCA ATA AAC CGT CAT GAG TAA TTA TTA CCA CTG TCC CAA
 <P S N S P G V S E I R F S W D R K T I Q C Y E N I I T V P N
 -----E3L-----
 2420 * 2430 * 2440 * 2450 * 2460 * 2470 * 2480 * 2490 * 2500 *
 AGC ATC TTT CCA ATC AAT AAT TTT AGC CGG AAT AAC ATC ATC AAA AGA CTT ATG ATC CTC TCT CAT TGA TTT TTC GCG GGA TAC ATC
 TCG TAG AAA GGT TAG TTA TTA AAA AAA TCG GCC TTA TTG TAG TAG TTT TCT GAA TAC TAG GAG AGA GTA ACT AAA AAG CGC CCT ATG TAG
 <A D K W D I I K K A P I V D D F S K H D E R M S K E R S V D
 -----E3L-----
 2510 * 2520 * 2530 * 2540 * 2550 * 2560 * 2570 * 2580 * 2590 *
 ATC TAT TAT GAC GTC AGC CAT AGC ATC AGC ATC CGG CTT ATC CGC CTC CGT TGT CAT AAA CCA ACG AGG AGG AAT ATC GTC GGA GCT GTA
 TAG ATA ATA CTG CAG TCG GTA TCG TAG CGC GAA TAG GCG GAG GCA ACA GTA TTT GGT TGC TCC TCC TTA TAG CAG CCT CGA CAT
 <D I I V D A M A D A D P K D A E T T M F W R P P I D D S S Y
 -----E3L-----
 2600 * 2610 * 2620 * 2630 * 2640 * 2650 * 2660 * 2670 * 2680 *
 CAC CAT AGC ACT ACG TTG AAG ATC GTA CAG AGC TTT ATT AAC TTC TCG CTT CTC CAT ATT AAG TTG TCT AGT TAG TTG TGC AGC AGT AGC
 GTG GTA TCG TGA TGC AAC TTC TAG CAT GTC TCG AAA TAA TTG AAG AGC GAA GAG GTA TAA TTC AAC AGA TCA ATC AAC ACG TCG TCA TCG
 <V M A S R Q L D Y L A K N V E R K E M N L Q R T L Q A A T A
 -----E3L-----
 2690 * 2700 * 2710 * 2720 * 2730 * 2740 * 2750 * 2760 * 2770 *
 TCC TTC GAT TCC AAT GTT TTT AAT AGC CGC ACA CAC AAT CTC TGC GTC AGA AGC CTC GTC AAT ATA GAT CTT AGA CAT TT TTAGAGAGAA
 AGG AAG CTA AGG TTA CAA AAA TTA TCG CGG TGT GTG TTA GAG AGC CAG TCT TGC GAG CAG TTA TAT CTA GAA TCT GTA AA AATCTCTT
 <G E I G I N K I A A C V I E A D S R E D I Y I K S M
 -----E3L-----
 2780 * 2790 * 2800 * 2810 * 2820 * 2830 * 2840 * 2850 * 2860 * 2870 * 2880 *
 CTAACACAAC CAGCAATAAA ACTGAACTTA CTTTATCATT TTTTTATTCA TCATCCTCTG GTGGTTCGTC GTTTCTATCG AATGTAGCTC TGATTAACCC GTCATCTATA
 GATTGTGTTG GTCGTTATT TGACTTGGAT GAAATAGTAA AAAATAAGT AGTAGGAGAC CACCAACCGAG CAAAGATAGC TTACATCGAG ACTAATTGGG CAGTAGATAT
 -----E3L-----
 2890 * 2900 * 2910 * 2920 * 2930 * 2940 * 2950 * 2960 * 2970 * 2980 * 2990 *
 GGTGATGCTG GTTCTGGAGA TTCTGGAGGA GATGGATTAT TATCTGGAAG AATCTCTGTT ATTTCTCTGT YYYCATGTAT CGATTCGCTT GAAACATTAA GATTGGAAAG
 CCACTACGAC CAAGACCTCT AAGACCTCT CTCACCTATA ATAGACCTTC TTAGACACAA TAAAGGAACA AAAGTACATA GCTAACCGAA CATTGTAATT CTAACGCTT
 -----E3L-----
 3000 * 3010 * 3020 * 3030 * 3040 * 3050 * 3060 * 3070 * 3080 * 3090 * 3100 *
 TGCTCTAAAT TTGGGAGGCT TAAAGTGTG TTGCAACT CTACACGGT GTCTAACTAG TGAGGTTCTG TCAGCTGCTC TAGTTGAAAT CATCATCGG GTAGTATCCC
 ACAGAGATTAA ACCCTCCGA ATTCACAAAC AAACGTTAGA GATGTGCCA CAGATTGATC ACCTCCAAGC AGTCGACGAG ATCAAACCTTA GTAGTAGCCG CATCATAGG
 -----E3L-----
 3110 * 3120 * 3130 * 3140 * 3150 * 3160 * 3170 * 3180 * 3190 * 3200 * 3210 *
 TACTTTTACA GTTAGGACAC GGTGTTAGT ATTCTCTGTC GAGAACGTTA AAATAATCGT TGTAACCTAC ATCCTTTATT TTATCTATAT TGTATTCTAC TCCCTTCTA
 ATGAAAATGT CAATCCTGTG CCACATAACA TAAAGGACAG CTCTTGCAT TTTATTAGCA ACATTGAGTG TAGGAAATAA ATAGATATA ACATAAGATG AGGAAAGAAT
 -----E3L-----
 3220 * 3230 * 3240 * 3250 * 3260 * 3270 * 3280 * 3290 * 3300 * 3310 * 3320 *
 ATGCATTTA TACCGAATAA GAGATAGCGA AGGAATTCTT TTTATTGATT AACTAGTCAAA ATGAGTATAT ATAATTGAAA AAGTAAAATAA TAAATCATAT AATAATGAAA
 TACGTAAAAT ATGGCTTATT CTCTATCGCT TCCCTAAGAA AAATAACTAA TTGATCAGTT TACTCATATA TATTAACCTT TTCAATTAT ATTAGTATA TTATTACCTT

Fig. 8 (cont'd)

3330	3340	3350	3360	3370	3380	3390	3400	3410	3420	3430
CGAAATATCA	GTAATAGACA	GGAACGGCA	GATTCTCTT	CTAATGAAGT	AAGTACTGCT	AAATCTCAA	AATTAGATAA	AAATGATACA	GCAAATACAG	CTTCATTCAA
GCTTTATAGT	CATTATCTGT	CCTGACCGT	CTAAGAAGAA	GATTACTYCA	TTCATGACGA	TTTAGAGGTT	TTAATCTATT	TTTACTATGT	CGTTTATGTC	GAAGTAAGTT
3440	3450	3460	3470	3480	3490	3500	3510	3520	3530	3540
CGAAATTACCT	TTAATTTT	TCAGACACAC	CTTATTACAA	ACTAACTAAG	TCAGATGATG	AGAAAAGTAA	TATAAATTAA	ACTTATGGGT	ATAATATAAT	AAAGATTCAT
GCTTAATGGA	AAATTAAGA	AGTCTGTGTG	GAATAATGTT	TGATTGATTC	AGTCTACTAC	TCTTCATT	ATATTTAAAT	TGAATACCCA	TATTATATTA	TTTCTAAGTA
3550	3560	3570	3580	3590	3600	3610	3620	3630	3640	3650
GATATTAATA	ATTTACTAA	CGATGTTAAT	AGACTTATTC	CATCAACCCC	TTCAAACCTT	TCTGGATATT	ATAAAATACC	AGTTAATGAT	ATAAAATAG	ATTGTTAACG
CTATAATTAT	TAAATGAATT	GCTACAATTA	TCTGAATAAG	GTAGTTGGGG	AAGTTGGAA	AGACCTATAA	TATTTATGG	TCAATTACTA	TAATTTATC	TAACAAATTC
3660	3670	3680	3690	3700	3710	3720	3730	3740	3750	3760
AGATGTAAT	AATTATTTGG	AGGTAAAGGA	TATAAATTA	GTCTATCTT	CACATGGAAA	TGAATTACCT	AATTTAATA	ATTATGATAG	GAATTTTTA	GGATTTACAG
TCTACATTAA	TTAATAAACCC	TCCATTCTT	ATATTTAAAT	CAGATAGAAA	GTGTACCTT	ACTTAATGGA	TTATAATTAT	TAATACTATC	CTAAAAAAAT	CCTAAATGTC
3770	3780	3790	3800	3810	3820	3830	3840	3850	3860	3870
CTGTTATATG	TATCAACAAT	ACAGGCAGAT	CTATGGTTAT	GGTAAACAC	TGTAACGGGA	AGCAGCATTTC	TATGGTAACT	GGCCTATGTT	TAATAGCCAG	ATCAJTTTAC
GACAATATAC	ATAGTTGTTA	TGTCGTCTA	GATACCAATA	CCATTGTTG	ACATTGCCCT	TCGTCGTAAG	ATACCATTGA	CCGGATACAA	ATTATCGTC	TAGTAAAATG
3880	3890	3900	3910	3920	3930	3940	3950	3960	3970	3980
TCTATAAAC	TTTACCAACA	AATAATAGGA	TCCTCTAGAT	ATTTAATAT	ATATCTAAC	ACAACAAAAA	AATTTAACGA	TGTATGGCCA	GAAGTATTTT	CTACTAATAA
AGATATTG	AAAATGGTG	TTTATATCT	AGGAGATCTA	AAATTATAAA	TATAGTTG	TGTTGTTTT	TTAAATTGCT	ACATACGGT	CTTCATAAAA	GATGATTATT
3990	4000	4010	4020	4030	4040	4050	4060	4070	4080	4090
AGATAAGAT	AGTCTATCTT	ATCTACAAGA	TATGAAGAA	GATAATCATT	TAGTAGTAGC	TACTAATATG	GAAGAAAATG	TATACAAAAA	CGTGGAAAGCT	TTTATATTAA
TCTATTCTA	TCAGATAGAA	TAGATGTTCT	ATACTTCTT	CTTATTAGTAA	ATCATCATCG	ATGATTATAC	CTTTCTTAC	ATATGTTTT	GCACCTTCGA	AAATATAATT
4100	4110	4120	4130	4140	4150	4160	4170	4180	4190	4200
ATAGCATATT	ACTAGAAGAT	TTAAATCTA	GACTTAGTAT	AACAAACAG	TTAAATGCCA	ATATCGATTC	TATTTTCAT	CATAACAGTA	GTACATTAAT	CAGTGATA
TATCGTATAA	TGATCTCTA	ATTTTAGAT	CTGAATCATA	TTGTTTGTG	AAATTACGGT	TATAGCTAAG	ATATAAGTA	GTATTGTCAT	CATGTAATTA	GTCACTATAT
4210	4220	4230	4240	4250	4260	4270	4280	4290	4300	4310
CTGAAACGAT	CTACAGACTC	AACATATGCAA	GGAAATAAGCA	ATATGCCAAT	TATGTCTAAAT	ATTTAACCTT	TAGAACTAAA	ACGTTCTACC	AATACTAAAA	ATAGGATAAG
GACTTGCTA	GATGTCGTAG	TTGATACGTT	CCTTATTCTG	TATACGGTTA	ATACAGATTA	AAATTATGAA	ATCTTGATTT	TGCAAGATGG	TTATGATTTT	TATCCTATGC
4320	4330	4340	4350	4360	4370	4380	4390	4400	4410	4420
TGATAGGCTG	TTAAAGCTG	CAATAAATAG	TAAGGATGTA	GAAGAAATAC	TTTGTCTAT	ACCTTCGGAG	AAAAGAACTT	TAGAACAACT	TAAGTTAAAT	CAAACCTTGTA
ACTATCCGAC	ATTTTCGAC	GTATTTATC	ATTCCTACAT	CTTCTTATG	AAACAAGATA	TGGAAGCCTC	CTTCTTGA	ATCTTGTGA	ATTCAAATTA	GTTCGAACAT
4430	*	*	*	*	*	*	*	*	*	*
TTTATGAAGG	TACC	AAATACTTCC	ATGG							

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/02669

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/64, 15/67, 15/86; A61K 48/00
 US CL :424/93.2, 199.1; 435/69.1, 172.3, 320.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 199.1; 435/69.1, 172.3, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS; DIALOG DATABASES: MEDLINE, BIOSIS, CA SEARCH, WORLD PATENT INDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,738,922 A (HASELTINE et al.) 19 April 1988, see entire document.	1-14, 18-26
A	PARK, H. et al. TAR RNA-binding protein is an inhibitor of the interferon-induced protein kinase PKR. Proc. Natl. Acad. Sci. USA. May 1994, Vol. 91, pages 4713-4717. See entire article.	1-14, 18-26

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z"	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

02 APRIL 1998

Date of mailing of the international search report

20 JUL 1998

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